

Automated Tube Furnace Combustion for Liquid Scintillation Sample Preparation

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INTRODUCTION

Almost since the first use of the liquid scintillation counting technique as a routine method for the measurement of Hydrogen-3 and Carbon-14 activities in biological materials, the potential value of combustion as a part of the sample preparation process has been evident. Combustion converts all such test substances to just two species — HTO and $^{14}\text{CO}_2$. If they can be solubilised in suitable counting solutions — and, of course, they can — then the problems of the investigator are obviously simplified. The combustion products are colourless and colour quenching is eliminated. Only one chemical species of each isotope need be counted; even if the counting mixture does exhibit chemical quenching, counting efficiencies should be near constant. The requirement for various counting mixtures and complex (and expensive) solubilisation techniques is eliminated; only two well defined and easily soluble combustion products need be handled. Materials that contain both isotopes give combustion products that are easily separated; dual labelling, with its complications of instrument settings, statistics and interpretation of results, is essentially eliminated.

But, sometimes reality does not parallel theory. While all of these advantages of combustion have long been appreciated, and have in fact been attained on a small scale, with few exceptions it has never been possible to achieve them with the ease required when many hundreds of samples must be processed. Until recently, the combustion method of choice has been that of Schoniger¹ as practised on samples of limited size² or even on a micro scale.³ With much manual effort, huge amounts of glassware, and dedication, a few busy laboratories have managed to keep up with their sample processing requirements via Schoniger combustion, but these are the exceptions. For the most part, conventional solubilisation methods are practised because combustion techniques have not offered the throughput necessary to satisfy normal needs. Until recently, none of the several combustion methods of the past,⁴ almost all of which give excellent results for a few samples, have been converted to the automatic or semi-automatic basis which might make such methods attractive to the typical laboratory.

Recognising that the problem was not fundamental, but only one of selecting the most suitable existing means and then automating that, a group at the National Institutes of Health, Bethesda, Md. under J.I. Peterson evaluated existing combustion methods with a view to choosing that most suitable for liquid scintillation sample preparation. They concluded that vertical tube furnace combustion^{5,6} offered the most promise and devised an improved system^{7,8} in which substances containing Hydrogen-3 and/or Carbon-14 can be combusted and the combustion products, trapped in appropriate counting fluids, automatically collected in counting vials which need only be manually capped before being counted. An independent assessment of the Peterson system has been provided.⁹ Because it forms the basis for the Intertechnique Oxymat combustion system,^{1,0} discussed in detail in this chapter, the Peterson method will now be briefly described.

THE PETERSON SYSTEM

The two essential elements of the Peterson apparatus are illustrated in Fig. 1. On the left is the sample introduction mechanism, the furnace and the HTO trapping system; the $^{14}\text{CO}_2$ collection unit is pictured on the right. The test substance, dry, moist or even in aqueous solution or suspension, is weighed out in a combustible capsule. Gelatin capsules were originally employed, but more recently polycarbonate resin, less affected by water and not giving large amounts of undesirable nitrogen oxides when burned, has been substituted. The capsule is

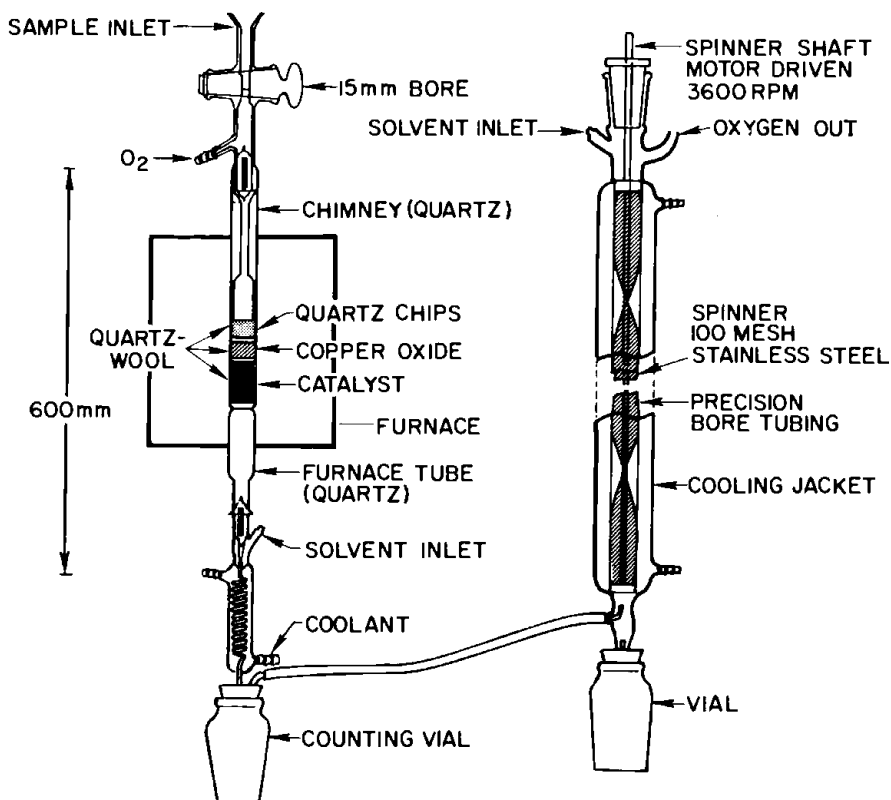


Fig. 1. The Peterson apparatus.

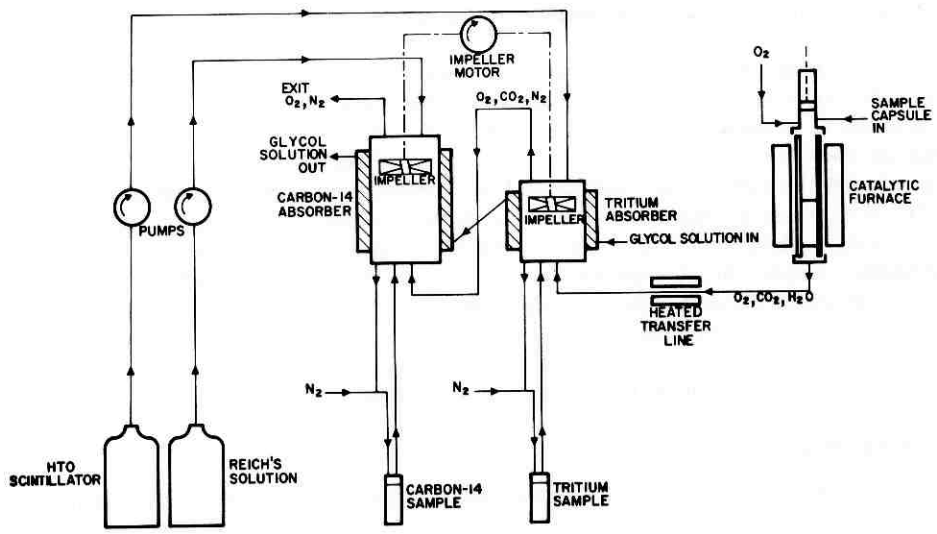


Fig. 2. Oxymat flow diagram.

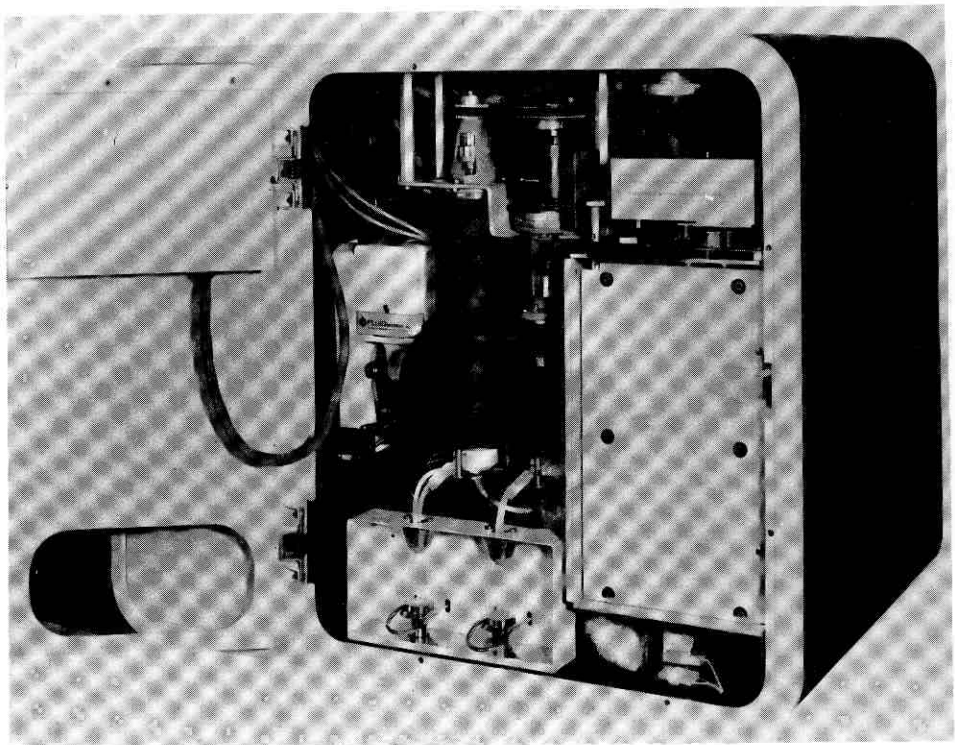


Fig. 3. The Oxymat. Product collection vials are in place; $^{14}\text{CO}_2$ is collected on the left, HTO on the right.

placed in the large diameter (15 mm) blind-bore stopcock on top of the quartz combustion furnace. Upon rotating the stopcock the capsule drops into the furnace on top of a bed of quartz chips where, at 600–650°C, and in a stream of oxygen flowing at 1 litre/min, the capsule bursts into flame. Volatiles are flashed off, surely some pyrolysis occurs, and finally the test substance burns. With sample size held to recommended limits – 1 g dry weight for Hydrogen-3, 0.5 g for Carbon-14 and no more than a total volume of 1 ml for aqueous solutions – combustion is over and there is no visible flame after 2 min. Combustion products, pyrolysis products and volatiles are swept downwards by the oxygen flow through a packed bed of copper oxide needles and hopcalite, a copper–manganese oxide catalyst. At the elevated temperature, and with the large oxygen reserve provided by both the flowing gas and the copper oxide bed, total oxidation is assured; only water vapour and CO₂ (together with nitrogen, rather than nitrogen oxides – possibly due to the catalyst) and, of course, excess oxygen, leave at the bottom of the furnace.

HTO, if present, is almost immediately trapped in a stream of liquid scintillator (methanol/toluene or dioxane/toluene with naphthalene, PPO and POPOP) which is introduced just below the bottom of the furnace. The scintillator stream, the combustion products and excess oxygen flow through an externally cooled coil and then into a counting vial. Scintillator flow rate is 10 ml/min for 2 min; HTO trapping is near quantitative. Once the scintillator flow stops and a few seconds are allowed for drainage, the vial may be removed for capping and counting. It has been shown, however, that counting efficiency is enhanced if the solution is bubbled for a few seconds with dry air or dry nitrogen; dissolved oxygen is removed; perhaps trace quantities of nitrogen oxides and more significant amounts of dissolved CO₂ are also.

If ¹⁴C₂O is to be trapped, whether or not HTO is present, the HTO counting vial must make a tight seal with the system so that no gases are lost. Non-condensed gases passing the HTO trap are directed to the ¹⁴C₂O trap where an alkaline scintillator solution (toluene/methanol/β-phenylethylamine with PPO and dimethyl-POPOP) sequesters CO₂. The CO₂ trap is a centrifugal wiped film absorber in which a high speed rotor (~3600 r.p.m.) assures maximum gas turbulence and creates a thin film of trapping solution with a view to presenting maximum surface area. External cooling takes up the heat of reaction between CO₂ and β-phenylethylamine and also, by maintaining reduced temperatures until the counting solution is in the vial, favours the reversible equilibrium towards increased carbamate formation. Once scintillator flow has ended – it usually begins a few seconds after the flow of the HTO scintillator would normally start and lasts about 30 s longer (but with about the same total fluid volume) – the vial is removed, capped and counted. For ¹⁴C₂O, air or nitrogen purging of dissolved oxygen is less important than for HTO but, nevertheless, is still useful.

THE INTERTECHNIQUE OXYMAT

A pictorial representation of the Oxymat is given in Fig. 2; photographs of the system and some of its key elements are shown in Figs. 3 and 4. The Oxymat is a direct descendent of the Peterson apparatus. As such, it retains its advantages and overcomes the disadvantages. It does, of course, include those refinements which are normally expected when laboratory apparatus is engineered into a commercial product.

Sample introduction is no longer by stopcock. Instead the self-standing 1.2 ml polycarbonate resin capsule is thrust into the vertical furnace through a port which opens when a heavy duty, spring-loaded solenoid lifts its stainless steel plunger

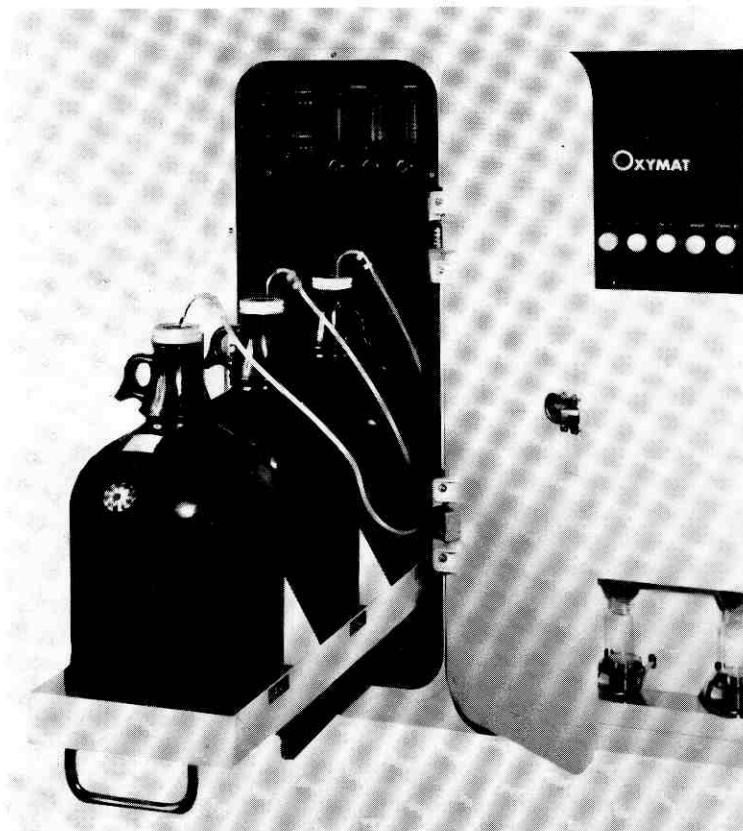


Fig. 4. With the Oxymat's right front panel open, the furnace compartment is at right. The heated transfer line, a part of which can be seen below the furnace, leads combustion products to the HTO absorber. To the left is the $^{14}\text{CO}_2$ absorber and then one of the two metering pumps.

from a Viton seat. The port is opened, capsule introduction takes place and the port is closed again in less than 3 s. The quartz tube furnace (operating at $650\text{--}700^\circ\text{C}$) remains vertically oriented but its packing (Fig. 5) has undergone considerable change from that of Peterson. Combustion of the capsule and its contents takes place on top of a bed of coarse silicon carbide chips. Below that, most of the packed zone is filled with 99% cupric oxide pellets (E. Merck, Darmstadt # 2768) which have an appreciably higher oxygen content and are less prone to sintering and clogging than the 80% $\text{Cu}_2\text{O}/20\%$ CuO needles formerly used. Finally, the small remaining lowermost zone is filled with a catalyst (W.R. Grace, Grade 908, Type SMR-7 35 35) of the type currently being evaluated for control of automobile exhaust pollution. The catalyst presumably eliminates nitrogen oxides in the product stream leaving the furnace. That nitrogen oxides are no problem is fact; whether or not the catalyst is really the cause of their elimination has not been adequately tested.

Combustion products leaving the furnace pass through a heated ($\sim 200^\circ\text{C}$) transfer line to the HTO absorber. Use of the transfer line allows the HTO absorber to be placed adjacent to the furnace rather than below it as in the Peterson apparatus. This makes for a better dimensioned system which allows the user easier access

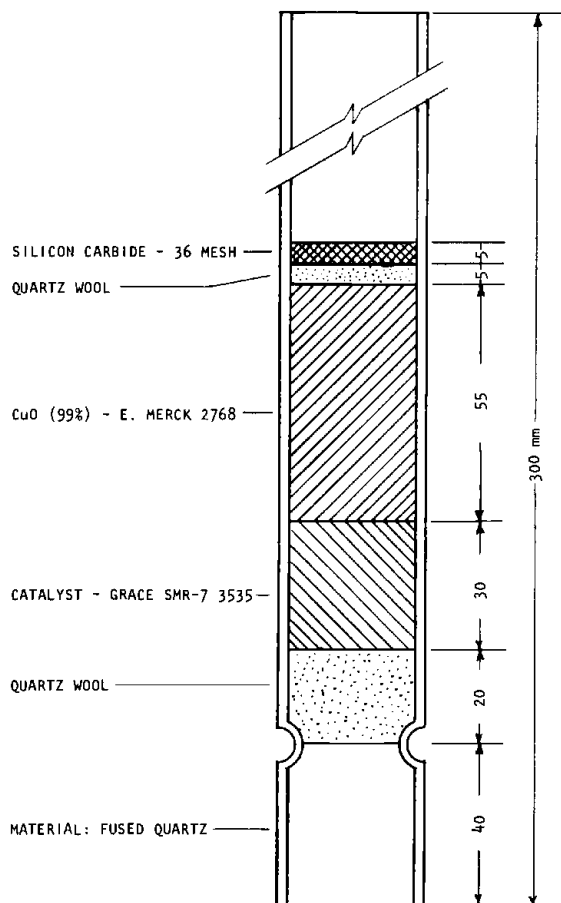


Fig. 5. Oxyamat combustion catalyst tube.

since it does not have excessive height. It also removes the absorber with its operating content of inflammable solvent from close proximity to the furnace and its flame.

Both the HTO and $^{14}\text{CO}_2$ absorbers use the centrifugal wiped film principle found so effective for $^{14}\text{CO}_2$ by Peterson. In this case, the $^{14}\text{CO}_2$ absorber is about twice as long as that used for HTO, and the $^{14}\text{CO}_2$ rotor spins at twice the speed, both in recognition of the greater difficulty of collecting $^{14}\text{CO}_2$. External cooling ($\sim 4^\circ\text{C}$) is employed for both absorbers and is provided by a circulating refrigerant system which is an integral part of the apparatus. Solutions leaving either of the absorbers are directed to counting vials which are held on spring-loaded platforms and make gas-tight seals to the unit via soft silicone rubber cones firmly fastened to the fluid outlets. But, before the scintillator solutions actually enter the counting vials they pass through nebulisers, one directly at the outlet of each absorber, where they meet a nitrogen purge. Deoxygenation takes place, as does CO_2 removal from the HTO solution. Without the latter, instances have been observed in which almost 5% of the $^{14}\text{CO}_2$ content from a dual-labelled sample came into the HTO vial. With the nitrogen purge, the maximum $^{14}\text{CO}_2$ content of the HTO vial

does not exceed 0.5% of the total $^{14}\text{CO}_2$ and is usually less.

The Oxymat incorporates those features and interlocks required of a commercial device. Adjustable timing cams allow precise turn-on and turn-off of the two stainless steel/Teflon controllable flow metering pumps (Fluid Metering Inc., Oyster Bay, New York) at the times most suitable for optimum product collection. Pressure sensors check the rates of oxygen and nitrogen flow and do not allow sample introduction if the rates are incorrect. Similarly, the temperatures of the furnace, transfer line and circulating refrigerant are monitored and operation is not possible if they are outside acceptable limits. Assuming adequate gas pressure, gas flows are automatically established; the operator is not required to manipulate valves other than to make certain that they are open. Temperature control is also automatic; the operator need not make any adjustments.

The Oxymat's combustion/collection cycle has a duration of 3 min; the next sample can be burned immediately after a cycle has been completed. Control is restricted to only a few pushbuttons. The operator denotes the nature of his sample — Hydrogen-3, Carbon-14 or Hydrogen-3/Carbon-14 — and after placing the combustion capsule with its test material on the introduction slide, depresses a single pushbutton. From there on, except for capping the vial, everything is automatic. The capsule is thrust into the furnace, combustion occurs, the pumps operate and trapping takes place, the nitrogen purge is carried out, and the counting vials are filled, all without operator assistance. In fact, during the 3 min combustion/collection cycle, the instrument may be left unattended, providing a good opportunity for the operator to weigh out or otherwise prepare the next sample. At the end of the day, or if the unit is not to be used for some hours, depression of the Wash pushbutton causes a methanol/toluene rinse to clean the system thereby preventing possible clogging of the fluid lines as solvent evaporates and scintillator is left behind.

OXYMAT PERFORMANCE

For the most part, the parameters that must be checked in evaluating the performance of a device such as the Oxymat are comparable to those for almost any analytical method. They are:

- (a) Precision.
- (b) Recovery.
- (c) Linearity with sample type.
- (d) Memory.
- (e) Linearity with sample size.

Other parameters particular to radioisotope measurement which must be examined include:

- (f) Counting efficiencies.
- (g) Dual isotope separation.

Each of these factors will be considered separately.

Precision

Table 1 provides results obtained on combustion of diluted whole blood labelled with ^{14}C -dihydroxyphenylalanine (DOPA). Twelve identical 200 μl samples were combusted sequentially; there was essentially no delay between the end of one combustion and the beginning of the next. Samples were counted twice; the average of the two counts of each sample was taken as the activity. The standard deviation of the twelve averages is seen to be of the same order as would be expected had the same quantity of activity been directly pipetted into a counting solution and

measured. Clearly, this suggests that Oxymat reproducibility is of a high order. Data presented below for other purposes supports this contention.

Table 1. Combustion of diluted whole blood.

Sample	1	2	Avg.
1	12313	12405	12359
2	12175	12206	12100
3	12345	12067	12206
4	12006	12125	12065
5	11737	11793	11765
6	12179	12191	12185
7	11903	12067	11985
8	11968	11866	11917
9	12095	12278	12186
10	12075	11874	11974
11	12112	11973	12042
12	12030	12139	12084
			Mean = 12072 ± 154.7

Recovery

Recovery measurements are based upon combustion of known amounts of activity. Tables 2 and 3 indicate better than 97% radioisotope recovery for combustion of bovine muscle homogenates to which known amounts of ^3H - or ^{14}C -cholesterol had been added. Such performance is typical and has been observed both by ourselves and our users over more than 20,000 combustions covering test substances of all types.

Table 2. Combustion of bovine muscle homogenate (^3H -cholesterol).

	Sample					Mean
	1	2	3	4	5	
c.p.m.	103186	111607	115337	116869	115475	112494 ± 4.94%
d.p.m.	275898	289137	279944	276940	276255	279634 ± 1.98%
Average recovery — 97.9%						

Table 3. Combustion of bovine muscle homogenate (^{14}C -cholesterol).

	Sample					Mean
	1	2	3	4	5	
c.p.m.	401.2	405.9	408.3	399.2	396.8	402.3 \pm 1.18%
d.p.m.	658.8	665.9	669.5	654.4	651.7	661.7 \pm 0.90%
Average recovery — 96.0%						

Linearity with sample type

That performance does not change with differing substrates is suggested by Table 4. Here, identical quantities of ^{14}C -cambendazole were added to 300 μl of various tissue homogenates or sucrose solution. Again, recoveries of activity were above 97%. There were no apparent differences related to the material being burned. With a mean count rate of 484.5 c.p.m. and a standard deviation of 12.05 for a total of 19 samples, it surely must be concluded that combustion considerably reduces the variability between sample types normally observed when conventional solubilisation procedures are employed.

Table 4. Combustion of ^{14}C -cambendazole — c.p.m.

Liver	Fat	Kidney	Lung	Muscle	Sucrose
488	481	487	482	479	497
484	491	489	472	510	496
471	498	485	454	480	
486					
476					
Mean of 19 samples — 484.5 \pm 12.05					

Memory

In collecting the data for Tables 5 and 6, blanks were burned between samples and the amount of activity from one sample that might have found its way into the next had there been no blank ('memory') was determined. The average of the 10 blanks for each isotope, expressed as a percentage of the preceding activity, is given together with the standard deviation.

Unfortunately, memory decrease does not follow a straight line function. A second blank burned immediately after the first typically exhibits 20% of the activity of the first or about 0.1% of the activity of the substance combusted.

Memory decreases as the non-use time interval after a combustion is lengthened, probably due to the slow release of activity from binding sites as a result of temperature, continued oxygen flow and possibly further drainage of the last few drops of scintillator solution from the absorbers. Within perhaps 30 min essentially all memory has been eliminated. Preliminary experiments with a brief steam purge immediately after combustion suggest that memory can be reduced by better than a factor 5, but it is presently premature to state whether or not the effect is long

term or if such a purge will prove practical in actual use.

Linearity with sample size

The effect of increasing sample weight on Oxymat performance, while still keeping within the suggested limits of 0.5 g for Carbon-14 and 1.0 g for Hydrogen-3, with the volume of aqueous solution not to exceed 1.0 ml under any circumstance, is less obvious than one might imagine. HTO counting efficiencies (see below) are dependent upon the quantity of water that must be trapped. Observed count rates, of themselves, do not have great significance and must be corrected for counting efficiency. When that is done, it is apparent (Table 5) that recovery is unaffected by sample size.

Table 5. Recoveries versus sample weight (Hydrogen-3).

	Butter mg		Fat mg		Liver mg		Testes mg		Whole blood mg	
	100	200	250	500	250	500	250	500	250	500
Recovery %	98.6	96.3	95.0	96.6	100.8	96.6	101.2	97.0	100.7	98.5
Memory %	0.37	0.37	0.26	0.19	0.21	0.93	0.45	0.05	0.29	0.18
Mean of 10 measurements: Recovery — $98.13 \pm 2.18\%$										
Memory — $0.33 \pm 0.24\%$										

The problem of collecting $^{14}\text{CO}_2$ is more difficult than collection of HTO where a physical process is so largely responsible. For that reason, maximum quantities of Carbon-14-labelled substances are limited. Again, observed count rates vary with the quantity of product collected, but here, because the β -phenylethylamine is more of a quencher than is its reaction product with CO_2 , the count rate per unit sample weight increases with increasing sample size. However, as shown in Table 6, when corrections are made for counting efficiency, there is no significant variation in recovery as sample size is altered.

Table 6. Recoveries versus sample weight (Carbon-14).

	Butter mg		Liver mg		Muscle mg		Testes mg		Whole blood mg	
	50	100	250	500	250	500	250	500	250	500
Recovery %	98.7	99.0	105.2	98.4	100.1	101.0	100.5	97.8	101.0	101.9
Memory %	0.35	0.35	0.40	0.25	0.25	0.38	0.34	0.38	0.61	0.25
Mean of 10 measurements: Recovery — $100.36 \pm 2.15\%$										
Memory — $0.36 \pm 0.11\%$										

Counting efficiencies

Counting efficiencies observed for scintillator solutions leaving the Oxymat are functions of solution composition, the quantity of combustion products trapped and, of course, the counter in which they are measured. Formulations have been

chosen to give the best compromise between trapping capacity, physical characteristics (especially viscosity), stability, absolute counting efficiency and constancy of counting efficiency with change in the concentration of trapped combustion products. The HTO solution — 700 ml dioxane, 300 ml toluene, 20 g naphthalene, 7 g butyl-PBD — up to its maximum useful water content of about 6% gives relatively good efficiencies when compared to more usual water counting preparations at the same water concentrations. In a modern counter, and with a non-quenching internal Hydrogen-3 standard, 40% counting efficiency should be expected. Data for increasing quantities of water produced by combustion of increasing quantities of sucrose are given in Table 7.

Table 7. Combustion of increasing sample weights (Hydrogen-3).

	Sucrose (mg)			
	248	350	450	553
Hydrogen-3 eff. %	37.6	35.8	35.4	33.3

While the HTO trapping solution is, for a solution of its type, relatively free from quenching, the same is not true of the $^{14}\text{CO}_2$ solution — 330 ml β -phenylethylamine, 220 ml methanol, 400 ml toluene, 50 ml water, 7 g butyl-PBD. With its high contents of β -phenylethylamine and methanol, and its rather low toluene content, it is highly quenched. However, since it is used only for Carbon-14, this is not a serious problem and, in fact, may be an advantage since the small amount of Hydrogen-3 contamination (see below) does not count. Counting efficiency for an unquenched Carbon-14 internal standard is typically about 75% and varies little with increasing quantities of $^{14}\text{CO}_2$ (Table 8).

Table 8. Combustion of increasing sample weights (Carbon-14).

	Salicylic acid (mg)			
	70	140	280	420
Carbon-14 eff. %	73	75	75	71

Dual isotope separation

Dual isotope separation is best measured by combusting singly labelled materials under dual isotope conditions. The two pairs of solutions so produced are counted, first in a wide window to ascertain the maximum amount of product collected in the absorber intended for the other isotope and then in the narrower window that is normally used to eliminate unwanted cross-contribution. In the first instance about 0.5% of each isotope comes into the vial for the other. When Carbon-14 contaminates the HTO vial, proper window setting (rejection of all counts above the Hydrogen-3 spectrum) will eliminate at least a third, and probably more, of the undesirable Carbon-14 counts. For HTO in the $^{14}\text{CO}_2$ vial, the situation is even better; the counting mixture itself serves to suppress the Hydrogen-3 counts making careful spectrometry largely unnecessary.

USER EXPERIENCE

It is difficult for the instrument manufacturer to anticipate all of the test substances likely to be encountered in the biomedical laboratory which various investigators might wish to subject to combustion. Therefore, it was of interest to us to inquire of our users as to their experiences with the Oxymat. Some representative examples are cited.

Combustion of Rat Faeces

In an experiment extending over a 30-day period, the daily excretion of ^{14}C -cholesterol in faeces was measured for 48 rats. A single collection from each animal was made daily and a representative sample of the pooled faecal material was counted after suitable processing. Initially, serious problems were encountered in obtaining uniformity and duplicates processed by combustion and by other means did not exhibit sufficient agreement. The difficulty was overcome by digestion of the pooled material with alcoholic KOH in capped, cone-bottomed tubes at about 85°C for 24 h. The digest was then neutralised (otherwise the alkalinity was found to attack to Lexan combustion capsule), agitated by hand to disperse remaining solids, and then duplicate aliquots were pipetted directly into the Lexan capsules. Activity after digestion was thought to be only in the liquid phase; solid particles were pipetted into the capsule only with the view of obtaining an average sample representative of the entire day's collection.

Open capsules were allowed to air dry overnight. The dried residue was burned without difficulty; over the 30-day period an average of 120 samples was combusted each working day. The entire experiment involved almost 3000 samples including standards and blanks. After each 12 combustions the Oxymat was put through a wash cycle to remove water which accumulated in the HTO trap and which, if not removed, led to the appearance of colour within the system. Ash was removed from the combustion furnace after each 200 samples.

Counting was performed with a Packard 3380/544. Inherent counting efficiencies were high but, by virtue of the 'electronic quenching' system of the unit,¹¹ it was not possible to know the maximum. Overall recovery for Carbon-14, as indicated by the combustion of commercially available plastic combustion standards (New England Nuclear Corp.), was 97% or better. Some of the experimental results are given in Table 9.

Table 9. Combustion of faecal samples — day 10.

Rat	ESR*	Counts/5 min	d. p. m.	Rat	ESR*	Counts/5 min	d. p. m.
1	0.2962	38974	9904	7	0.3025	60856	15493
1	0.3006	39565	10055	7	0.3004	59191	15068
2	0.3035	42620	10835	8	0.3054	19787	5003
2	0.3065	41436	10533	8	0.2935	21440	5425
3	0.3021	29648	7522	9	0.2980	30014	7615
3	0.2994	32681	8297	9	0.2953	31335	7953
4	0.3032	33238	8439	10	0.3020	18781	4746
4	0.3056	35963	9128	10	0.3100	19655	4969
5	0.2923	21392	5413	11	0.3048	25606	6489
5	0.3041	24284	6152	11	0.3051	30378	7708
6	0.3027	48201	12261	12	0.2969	33674	8550
6	0.3014	48041	12220	12	0.1982	31148	8932

* External Standard Ratio

Table 10. Activity distribution in a whole animal (Carbon-14).

Organ	ESR * ^a	Wet wt. ^b	d. p. m.	d. p. m./g	Total d. p. m.
Liver	0.3034	0.223	3084	13831	94355
"	0.3029	0.235	3158	13441	91693
Pancreas	0.3032	0.249	1041	4181	2989
Kidney	0.3084	0.188	936	4981	6994
"	0.1983	0.189	863	4567	6412
Adrenal	0.1977	0.033	163	4958	317
Spleen	0.2031	0.265	744	2810	1284
Ovary	0.2995	0.065	300	4618	591
Uterus	0.2034	0.187	872	4665	2286
Bladder	0.3030	0.050	113	2266	227
Lung	0.1959	0.174	476	2737	2893
"	0.3028	0.163	430	2639	2790
Adipose	0.2969	0.089	824	9260	1519
Muscle	0.2010	0.224	122	546	338
Brain	0.1957	0.246	77	312	535
"	0.2006	0.257	74	286	489
Heart	0.1995	0.288	435	1512	1034
Pituitary	0.3042	0.005	15	2920	15
Stomach	0.3003		10	1425	2251
"	0.3043		13	1324	2093
S. intestine	0.3002		582	2746	8923
"	0.2991		644	2709	8801
L. intestine	0.3007		482	47781	61446
"	0.2960		502	54398	69956
S. int. wash	0.2998		1117	364	10198
"	0.3037		1019	374	10478
L. int. wash	0.3046		1678	79131	1820008
"	0.3096		1487	81298	1869845
Stomach wash	0.3032		28	760	2910
"	0.3002		22	561	2577
Faeces					5431088
Urine					6050000
Serum					22866
Injected dose					15147000 = 89.5% Recovery

* External Standard Ratio.

^a 0.3 = 79% eff.

0.2 = 70% eff.

^b Dry faeces was combusted.

Total activity distribution in an animal

A 1, 5-¹⁴C six carbon carbohydrate was administered to female Charles River rats by oral intubation; the dose was approximately 1.5×10^7 d.p.m. per animal. At intervals after administration animals were dispatched; blood was collected, urine and faeces were recovered, stomach contents were segregated and organs were excised from the carcass. Representative samples of all materials were combusted and the total radioactivity content of each part of the body was determined. All tissue samples and other materials, with the exception of faeces, were burned wet; faeces were lyophilised and pulverised prior to combustion. The quantity of material combusted in each case was approximately 0.2 g or 0.2 ml; the pituitary, adrenals and ovaries were oxidised in entirety.

Counting of the products of combustion with an Oxymat was performed in a Packard 3380/544. Because of the nature of the instrument operation, it was not possible to know the inherent counting efficiencies of the solutions that were counted. However, only two external standard ratios were observed, one corresponding to approximately 79% efficiency and the other to 70%. As the particular instrument in question is known to frequently 'drop through' a high external standard ratio to a lower one, it is presumed that all samples had a value greater than 79%. Results for a single animal are given in Table 10.

Combustion of plant leaf extracts

Leaves taken from plants grown in the presence of Hydrogen-3/Carbon-14 doubly labelled compounds were homogenised with acetonitrile. Solids were discarded and the intensely coloured solution was collected for counting. Combustion of the neat acetonitrile solution proved unsuccessful due to extreme vigour and rapidity. However, when combustion was retarded with the aid of cellulose powder, quite satisfactory results were obtained. In each of the experiments cited in Table 11, 100 mg of cellulose powder was added to the combustion capsule before the acetonitrile solution was introduced. Blanks, used to check memory after each five samples contained only cellulose.

Experimental data is provided for five replicates of each of three different acetonitrile volumes. Within each group, precision is quite acceptable. Unfortunately, though external standard ratios indicate slight differences in counting efficiency as more combustion product is collected, corrections for quenching were not made. Therefore it is not surprising that the relationship between count rate and volume is not quite linear being 1.944:1 and 1.952:1 for Hydrogen-3 and Carbon-14 respectively rather than the expected 2:1 and 3.806:1 and 3.613:1 instead of the anticipated 4:1. However, when the Hydrogen-3:Carbon-14 ratios are examined, they are seen to be almost identical in all three groups, an indication that the degree of change in quenching with product concentration is about the same for the two isotopes in the weight range employed.

Combustion of polyacrylamide gel slices

A bacterial protein into which ¹⁴C-isoleucine had been incorporated was subjected to gel electrophoresis. After staining and measurement of the bands, the gel cylinder was cut into 1 mm segments which were placed in combustion capsules and burned in an Oxymat. Counting was performed in an Intertechnique SL30; channels ratio was used as an index of counting efficiency since the counting data was further processed by computer using a program based on this method of quench correction. Counting data from a typical experiment is presented in Table 12. Counting efficiency is seen to be essentially constant; on examination of those

Table 11. Combustion of plant leaf extracts.

Sample	ml	Hydrogen-3		Carbon-14		$^3\text{H}/^{14}\text{C}$
		c. p. m.	ESR*	c. p. m.	ESR*	
1	0.05	4288	6.98	2043	2.63	2.10
2	0.05	4295	7.17	2155	2.65	1.99
3	0.05	4012	6.57	2057	2.61	1.95
4	0.05	3992	6.73	2032	2.46	1.96
5	0.05	4227	7.12	2128	2.65	1.99
Mean		4163	6.91	2083	2.60	2.00
Std. dev. %		3.58	3.72	2.64	3.08	2.93
Blank		6(0.14%) ^a	7.17	10(0.48%) ^a	2.81	
6	0.1	8072	6.90	4078	2.54	1.98
7	0.1	8119	6.86	4118	2.52	1.97
8	0.1	8030	6.52	4083	2.51	1.97
9	0.1	8019	6.64	3932	2.45	2.04
10	0.1	8230	6.90	4131	2.55	1.99
Mean		8094	6.76	4068	2.51	1.99
Std. dev. %		1.06	2.57	1.95	1.56	1.46
Blank		28(0.34%) ^a	7.26	18(0.44%) ^a	2.80	
11	0.2	15335	6.35	7304	2.26	2.10
12	0.2	15976	6.59	7045	2.47	2.27
13	0.2	16110	6.54	7754	2.46	2.08
14	0.2	15806	6.51	7898	2.44	2.00
15	0.2	16002	6.64	7634	2.39	2.10
Mean		15846	6.53	7527	2.40	2.11
Std. dev. %		1.93	1.69	4.61	3.59	4.63
Blank		59(0.37%) ^a	7.26	55(0.58%) ^a	2.81	

* External Standard Ratio

^a Memory %

channel ratio values that seem to be aberrant, it is evident that the number of counts above background totally accumulated were too low for the ratios to be meaningful.

Table 12. Combustion of polyacrylamide gel slices (Carbon-14).

Slice	Gross count	Time	Net c.p.m.	Ch. ratio
1	20038	3.89	5128	1.344
2	20006	8.75	2263	1.364
3	20000	17.38	1127	1.495
4	20008	18.13	1080	1.489
5	585	20	6	2.038
6	20007	10.10	1958	1.453
7	783	20	16	1.710
8	20010	9.65	2050	1.510
9	20014	6.01	3307	1.490
10	20007	7.29	2721	1.651
11	9772	20	465	1.526
12	20033	6.73	2953	1.511
13	20002	5.46	3640	1.542
14	20020	4.56	4367	1.545
15	20036	4.53	4400	1.598
16	20033	5.93	3355	1.724
17	20016	9.01	2198	1.491
18	20008	18.34	1068	1.661

Miscellaneous

Several potentially interesting applications have been reported to us, but with insufficient experimental data for them to be described in detail. They are cited here, however, to suggest that the scope of the combustion method is broader than treatment of biological material:

- (1) In a health physics screening programme, the possible content of Hydrogen-3 and Carbon-14 in urine is separated from natural Potassium-40 by combustion in an Oxymat.
- (2) In column chromatography where recoveries are not quantitative, samples of ion-exchange resin from along the column length have been burned to locate bands of activity.
- (3) Labelled soil samples are being burned. Due to the high ash content, the catalyst tube must be cleaned after each 20 combustions. Though such frequent cleaning is rather time consuming, no other method previously examined has allowed these same samples to be counted.

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DISCUSSION

B.E. Gordon: How important is the Hopcalite or other CO oxidiser? Most train combustions use only CuO to get complete oxidation to CO₂.

E. Rapkin: We have never made use of Hopcalite due to our inability to obtain it on a commercial scale. We do use an automobile exhaust emission control catalyst because it seems logical to ensure complete conversion of CO to CO₂ and nitrogen oxides back to N₂. As we have gained experience with the Oxymat, our tendency has been to reduce the volume of catalyst and increase the bed of copper oxide with small, but noticeable, improvements in recovery and especially in reduction of memory. I suppose that inertia, together with the thousand or more samples that must be burned for a complete test, has kept us from evaluating the performance with copper oxide alone.

B. Scales: You rejected the use of gelatine capsules as containers for your radioactive samples because of unwanted combustion products. Does this mean that the combustion of certain raw biological samples can also give rise to undesirable combustion products, and can therefore cause difficulties?

E. Rapkin: We avoid gelatine because it has been reported that combustion of gelatine capsules leads to the formation of nitrogen oxides which, in Peterson's original apparatus, apparently found its way to the counting vials where it acted as a quencher. Since we could specify almost any plastic which we thought best as we were having capsules made to our requirements, we chose Lexan, a polycarbonate resin which contains no nitrogen and which avoids the problem if, in fact, there is one. With biological samples, we have never observed any problem from nitrogen oxides and we have never sought for the reasons as to why we don't have any problems. It may be that the nitrogen purge which we use, and which Peterson didn't, is enough to overcome any difficulties. It may also be that our automobile emission control catalyst breaks down nitrogen oxides.

A.R. Ware: In suggesting that combustion is a good replacement for conventional solubilisation techniques for biological samples, you are adding to the problem of measuring counting efficiency accurately that of measuring the percent of Carbon-14 and Hydrogen-3 from the original sample. I accept that the counting efficiency determination is much improved by this technique as near constant quenched samples are obtained. How often is calibration necessary in order to obtain an accurate recovery figure?

E. Rapkin: We advise our users to combust standard samples at least once each day the Oxymat is in use. If it is being used continuously throughout the day, we would like our users to burn standards morning and afternoon. A system with mechanical seals and a catalyst with a finite life (2-3000 samples) ought to be checked routinely as a matter of good laboratory practice. Combustion of a

standard requires only three minutes.

A.R. Ware: Does the recovery figure vary at all for different materials or is a single standard material adequate?

E. Rapkin: As long as the suggested weight restrictions are adhered to, recovery is near constant for all substances which we have tested so long as they do not burn explosively. Explosive combustion has never damaged any of the equipment but the rush of gaseous products produced thereby is too great for the trapping system. Combustion of standards is not suggested as a measure of the recovery of any particular substance but rather as a measure of the integrity of the apparatus and the efficacy of the copper oxide/catalyst section.