

Sample Preparation with an Automated Oxygen Flask Combustion Apparatus for Liquid Scintillation Counting of ^3H -, ^{14}C - and/or ^{35}S -labelled Material

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INTRODUCTION

Insoluble materials and quenching differences for soluble materials cause difficulties in sample preparation and thus diminish considerably the advantages of sensitive determination of weak β -emitters by means of liquid scintillation counting. In addition, measurement of insoluble materials in emulsions or suspensions rarely gives exact analyses, and the measurement of quenching from very different materials by one of the channel ratio methods, for example, easily leads to falsified results.

For organic or biochemical compounds, however, these difficulties can be overcome by combustion of the samples. From Hydrogen-3, Carbon-14 and Sulphur-35 labelled materials, one obtains uniformly HOT-, $^{14}\text{CO}_2$ - and oxides of Sulphur-35 respectively — singly or in combination. All these oxidation products are soluble in appropriate liquid scintillation systems, with defined and known quenching characteristics. A disadvantage of these procedures lies in the considerable manual labour associated with the combustions, irrespective of the particular method selected.

The first part of this paper, therefore, describes an automated apparatus, in which an oxygen flask combustion^{1,2} is carried out. This apparatus is commercially available with the designation 'Pyroszint'.

The second part of this paper attempts a comparison of advantages and disadvantages of the automated oxygen flask combustion and the oxygen stream technique.

APPARATUS

The automated apparatus consists of the combustion apparatus (Fig. 1), the shaking device (Fig. 2) and the racks. Each rack can hold eight normal Erlenmeyer flasks with a volume of 0.5 or 1 litre.

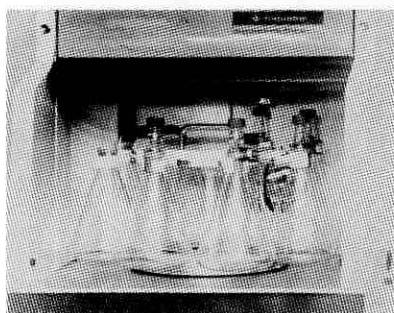


Fig. 1. Combustion apparatus.

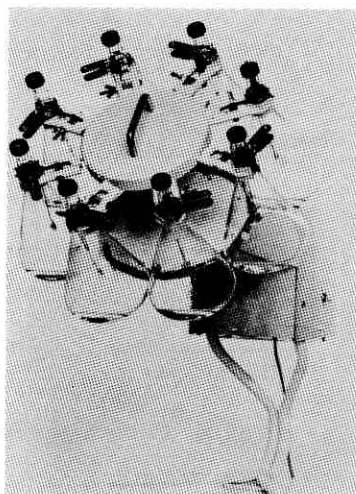


Fig. 2. Shaking device.

The combustion apparatus is equipped with a device for filling the flasks with oxygen, a projector lamp for the ignition and a dispenser for the exact delivery and injection of a scintillator-absorbent solution from a storage vessel. The combustion flask is closed manually by a sample holder (Fig. 3) which holds the filter paper-wrapped sample in a wire basket. The holder seals the flask by means of a screw cap, carrying a septum. Through this septum the scintillator-absorbent solution is automatically injected, after the combustion.

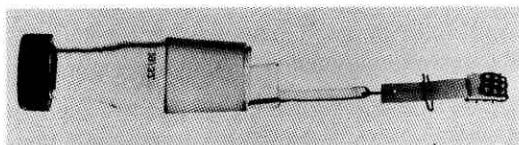


Fig. 3. Sample holder.

The actual procedure in detail is arranged as follows :

- 1 For solid samples, samples of up to 100 mg are weighed on ashless filter-paper cut-outs (Fig. 4). (These special cut-outs are commercially available.) For liquid samples, up to 100 μ l of aqueous aliquots can be applied directly to a filter tablet without drying.
- 2 The filter paper-wrapped samples or the filter tablets are mounted on the sample holders, which are securely equipped with septum and screw cap. The holders can then be stored in a rack (Fig. 5), until they are inserted into the combustion flasks.
- 3 A rack with eight Erlenmeyer flasks is then set into the combustion apparatus. The oxygen flush is initiated by push-button. Two flasks are flushed at the same time for 1 min, approximately, at an adjustable flow rate of about 2 litres/min. Immediately after the flush, the two flasks are closed with the prepared sample holders.

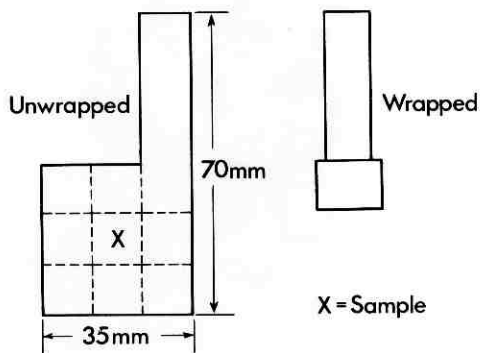


Fig. 4. Filter paper cut-out.

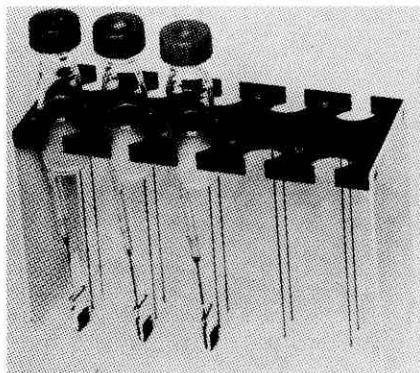


Fig. 5. Sample holder rack.

- 4 When all flasks are filled with oxygen and closed with sample holders, the push-button activates the ignition of the sample by means of a focussed beam from a projector lamp.
- 5 The apparatus then stops for 6 min for cooling after the last ignition. A defined volume of scintillator-absorbent solution is then automatically injected through the septum of the sample holder of each flask.
- 6 After the last injection, the rack is transferred to the shaking device – 10 min is needed for shaking.
- 7 An aliquot from each flask is then transferred to a counting vial. The sample is then ready for spectrometric measurement and evaluation.

A flow sheet procedure is shown in Fig. 6. It can be seen that approximately 5 min is needed for each sample. While the apparatus runs one batch of analyses, sufficient time is available to make weighings for the next group of samples.

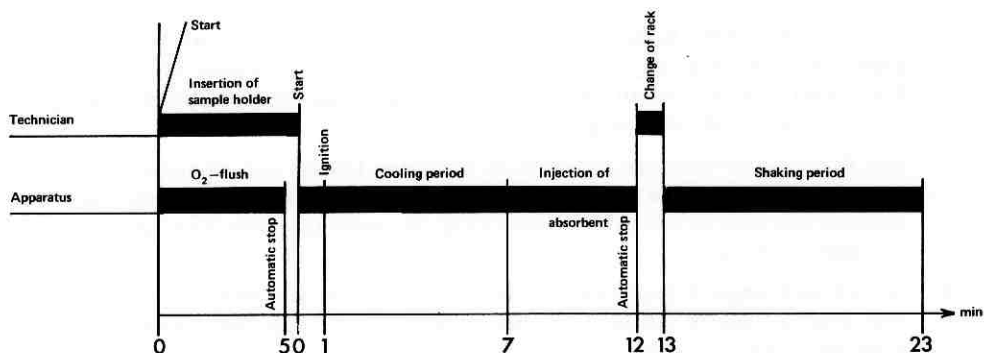


Fig. 6. Flow sheet of procedure.

RESULTS

The following scintillator-absorbent solution has proved to be sufficient:

180 ml	2-Phenylethylamine,
250 ml	Methanol,
570 ml	Toluene,
5 g	PPO (i. e. 2, 5-Diphenyloxazole),
and 0.5 g	Dimethyl-POPOP (1, 4-bis-2-(4-Methyl-5-phenyloxazolyl)-benzene).

This mixture is a modification of the one described by Woeller³ and it permits the simultaneous measurement of Hydrogen-3 together with Carbon-14 and of Hydrogen-3 together with Sulphur-35. For Sulphur-35 analyses, 4% of water has to be added to the mixture. In modern spectrometers counting efficiencies for singly labelled materials are 20% for Hydrogen-3 and 85% for both Carbon-14 and Sulphur-35, or, for doubly labelled materials, 16% for Hydrogen-3 and 50% for Carbon-14 or Sulphur-35, with a spillover of 10% Carbon-14 or Sulphur-35, respectively, into the Tritium counting channel.

Tables 1 and 2 show some results that were obtained with the apparatus. All samples are strong quenchers and/or insoluble. Each sample in a weight range of 10–100 mg was analysed 8–10 times and standard deviations of singly labelled materials of 1.0–2.1% have been obtained. Doubly labelled materials show standard deviations dependent on the isotope ratio. For a ratio Hydrogen-3/Carbon-14 of about 0.4 the deviations are 3.3–5.7% for Hydrogen-3 and 1.5–1.6% for Carbon-14. Isotope ratios of 4.5–8.7 show deviations of 1.2 and 1.0–2.9%, respectively.

Before continuing further, some of the advantages of the described procedure in comparison to the 'classical' flask combustion should be pointed out. The oxygen flush of the flasks, the ignition and the delivery of defined aliquots of scintillator-absorbent solution are completely automated. No cooling materials are needed whatsoever; singly-labelled, and either Hydrogen-3/Carbon-14 or Hydrogen-3/Sulphur-35 doubly-labelled samples can be analysed by a single procedure.

The sample combustion in streaming oxygen has also been automated,⁴⁻⁶ and some apparatuses are commercially available. Both procedures, flask and stream, have inherent advantages and disadvantages that are shown in Tables 3 and 4.

Table 1. Results for singly labelled materials.

Compound/nuclide	Number of samples	d. p. m./mg	% S.D.	% Recovery
Nicotinic acid / ³ H	8	10719	1.25	94.6
Nicotinic acid / ¹⁴ C	8	5303	0.99	95.2
Glucosazone / ³ H	9	3189	1.76	—
Glucosazone / ¹⁴ C	10	1513	1.24	—
p-Nitraniline / ³ H	9	10658	1.48	—
Liver / ³ H	8	1001	1.50	—
Faeces (rat) / ¹⁴ C	8	626	2.14	—
Thiourea / ³⁵ S	10	1481	1.90	100.9

Sample weight range, 10–100 mg.

Table 2. Results for Hydrogen-3/Carbon-14 doubly labelled materials.

Compound	No. of Samples	d. p. m. /mg		Isotope ratio (measured)	% S.D.	Isotope ratio (theoretical)
		$^3\text{H}/\%$ S.D.	$^{14}\text{C}/\%$ S.D.			
Nicotinic acid I	11	1990/3.26	4394/1.51	0.453	3.60	0.460
Nicotinic acid II	10	7512/1.05	1657/1.01	4.53	1.45	4.61
Glucosazone I	8	505/5.68	1240/1.60	0.407	5.90	-
Glucosazone II	10	2437/1.22	281/2.86	8.67	3.11	-

Sample weight range, 10-100 mg.

Table 3. Oxygen flask combustion (Pyroszint).

Advantages	Disadvantages
Without memory effects	No chemical separation of ^3H from ^{14}C ^a
Accuracy of results not influenced by chemical composition	For each combustion a flask has to be cleaned
Accuracy of results not changed by differing sample weights	Decreased sample number per time unit, as compared to stream technique
Procedural errors visually observable	Sample weights up to 100 mg
Minimal technical experience is needed	

^a Physical separation by the spectrometer is reliable up to $^3\text{H}/^{14}\text{C}$ ratios of ≥ 0.5 .

Table 4. Oxygen stream combustion

Advantages	Disadvantages
Increased sample number per time unit, as compared to flask technique	Memory effects up to 1 % ^a
Cleaning of combustion vessels unnecessary	Empty combustion decreases the background only asymptotically
Chemical separation of ^3H from ^{14}C	Accuracy of results influenced by the chemical composition
Sample weights higher than 100 mg are possible	Accuracy of results influenced by differences in sample weight
	Procedural errors not directly observable and can only be found with test runs
	Apparatus requires experienced technician

^a Following a sample with 10^5 c. p. m. a blank sample shows an activity of 10^3 c. p. m.

As was shown, the two analytical methods do not exclude or replace each other, but have special features which permit adjustment to the particular experimental problem that is at hand.

ACKNOWLEDGEMENT

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DISCUSSION

G. Stoydn: It is stated that 6 min cooling be allowed following the combustion in an oxygen flask. Is the period of cooling critical from the safety point of view and are there other considerations also involved?

P. Rauschenbach: The cooling period should not be smaller than 2 min. Under this time period there is a possibility of explosions, but the time of 6 min excludes securely this hazard.

C. Palais: What is the total volume of scintillator-absorber added to the combustion flask and what is the volume of the aliquot used for counting?

P. Rauschenbach: The total volume is 17.5 ml from the dispenser and the aliquot volume 15.0 ml from a pipette.

B.E. Gordon: Has anyone taken into consideration that the reaction of CO_2 + amine \rightleftharpoons carbamate is an equilibrium reaction which is temperature dependent? Thus quantitative recovery may not be achieved if the temperature is not low enough ($\approx 0^\circ\text{C}$).

P. Rauschenbach: In the work of Woeller {Anal. Biochem. **2**, 508 (1961)} there is no comment concerning a temperature dependence at room temperature, and we too could not observe such a dependence.