

OXI-FLO:
AN INSTRUMENT CONCEPT FOR THE COMBINED SAMPLE PREPARATION
AND MEASUREMENT OF LOW ENERGY BETA RADIO-LABELLED BIOLOGICAL
SAMPLES

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INTRODUCTION

Liquid scintillation counting (LSC) as a quantitative method for tracer methodology has a relatively long and distinguished past. Instrument manufacturers have reached the point in the development and progress of the technology where LSC is a routine analytical tool today. In their zeal to provide a simple, easy-to-use instrument, they have overlooked one problem. Insoluble and solid biological samples can give unflagged erroneous results, even using the most sophisticated liquid scintillation counters, due to sample preparation. This is a subject no one really cares to talk about: After paying upwards of \$25,000 for a sophisticated instrument, the results are expected to be correct all of the time.

One solution to the problem of sample preparation has been around for the past fifteen years, but for a number of reasons has not gained popularity.

SAMPLE OXIDIZERS

The theory behind sample oxidizers is simple. By burning practically any material in an atmosphere of oxygen, besides minute amounts of byproducts, the hydrogen (^3H) and carbon (^{14}C) content of the sample will yield water and carbon dioxide, both of which can easily and reproducibly be handled in any liquid scintillation counter.

A number of commercially produced oxidizers that somewhat automate the combustion of biological materials and yield LSC compatible samples have been around for years. The roots of the automated methods go back to the chemical Schonigen flask^{1,2,3} and Peterson's

oxidizer^{4,5,6,7}.

Samples are placed in a holding vessel, then transferred into either an ignition basket, or into a heated furnace where they dehydrate and burn in excess oxygen atmosphere; or, the products of combustion are swept through a heated catalytic exchange column where the oxidation process is completed. All oxidizers operate in a dynamic mode: the oxygen flow carries the products of combustion through the system. Water vapor is condensed and mixed with a nongelling organic scintillator and disposed of into an LSC vial. The carbon dioxide, carried with the gas stream into a secondary column, is absorbed in an amine which, together with the carbamate formed, is mixed with another type of organic scintillator and transferred into the LSC vial.

With minimal variations in technology, all the commercial oxidizers work in the above described manner^{8,9,10}.

The samples produced by oxidizers - having no optical quench and well-defined, predictable chemical quench - can be measured in any liquid scintillation counter, therefore, the use of an oxidizer allows the researcher to work with unsophisticated, therefore, inexpensive liquid scintillation counters.

Why then, is the use of oxidizers so limited and the technique a method of last resort? Three answers are the most obvious:

- a. Oxygen, high temperature, flame and organic solvents are present in all oxidizers and they are an "explosive" combination. Early models were prone to accidents and the potential hazard still discourages some users even though the technology today is advanced to the point where mishaps are almost nonexistent.
- b. The use of oxidizers requires continuous human attention. The preparation of samples, the combustion cycle, and the collection and capping of the LSC vials require the individual attention of an operator. An automatic sample oxidizer has long been requested by the public, but never delivered by the industry due to the many logistic problems and necessary safeguards required.
- c. The cost of oxidizers is high compared to other methods of sample preparation and compared to the cost of the liquid scintillation

counters. It is hard to justify similarly high costs for a counting instrument and an accessory which can be substituted by an inexpensive manual technique (sample solubilization), even though this alternative is less than perfect.

OXI-FLO

The purpose of the development of the OXI-FLO instrument was to overcome the objections raised above. It provides an automated system which will produce accurate, reproducible results at a cost which is competitive with current techniques, but which also provides savings in labor. The idea of producing an automatic oxidizer was rejected due to the difficulties associated with filling, capping and handling vials. An oxidizer, coupled with an LSC on the other hand, has many advantages. The technique of vial-less LSC was pioneered in this company, during the development of the RSP-B400 Sample Processor, therefore, the technology of transferring and counting a liquid in a stop-flow mode was readily available. The OXI-ONE Sample Oxidizer, also marketed by Radiomatic Instruments, lends itself naturally to the OXI-FLO concept, since the LSC vial is not an integral part of the oxidizer and can be substituted with a different device.

These two technologies combined are the basis of the OXI-FLO instrument. While not automatic in the true sense, the OXI-FLO forms the basis of a family of sample preparation/sample counting instruments envisioned in the future. The advantage of the current device is that sample preparation (oxidation) and sample counting (LSC) are performed simultaneously, but independently from each other. While one sample is being counted in its oxidized form, the next sample can be oxidized. Since a typical sample preparation cycle (dehydration, burn, collection, system clean-up) takes approximately four minutes, a four minute sample count can be simultaneously handled in the instrument, not only doubling the sample throughput, but also providing immediate results. While the counting efficiencies change with sample size, the relationship between the counting efficiency of the sample and the amount of water or CO₂ contained within is very simple, describing a direct shift in the energy spectrum, so the

counting channels ratios method of quench correction is well suited for immediate DPM calculations of the results.

Having no vials to contend with, the spacial requirement of the instrument is not more than that of a traditional oxidizer or a small bench top LSC alone, yielding a 50% savings in necessary laboratory space.

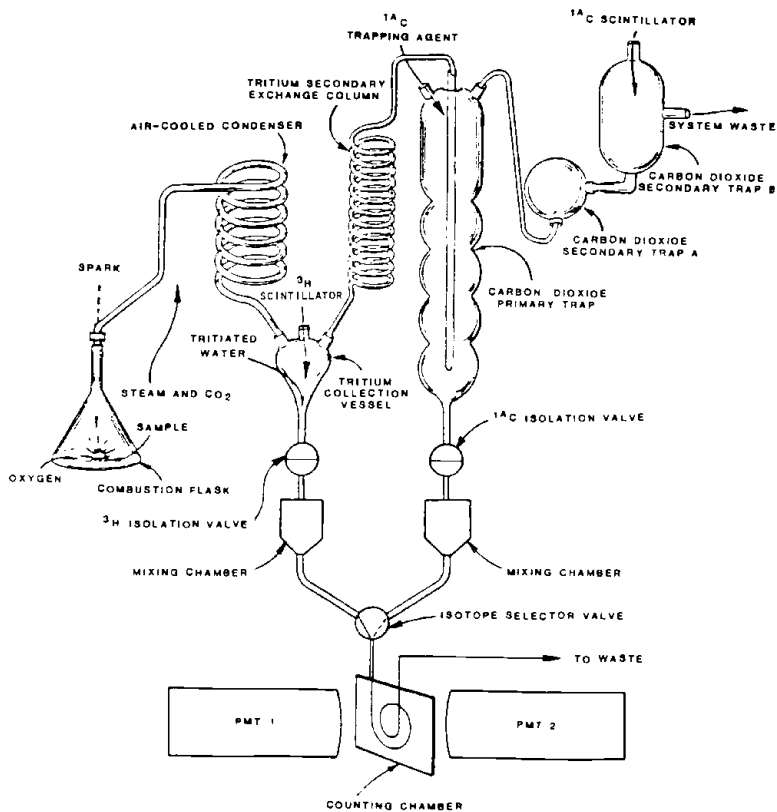


Figure 1. OXI-FLOW functional diagram

Figure 1 is a functional diagram of the original OXI-FLO system. Up to 0.5 g solid or liquid samples of combustible or noncombustible

materials can be placed either on a metal planchet, or in a porcelain crucible for processing. If the sample is unable to support combustion, a high boiling point organic solvent is used as a fuel to complete the oxidation of organic materials in the sample (e.g. soil samples). The planchet or crucible is elevated into a preheated combustion chamber where the sample is ignited by a high voltage spark source. The duration of the burn is manually controlled or preset to a desired time to assure complete combustion of the sample. The spark during combustion not only serves as an igniter, but continuously seeks out ionized gases and assures the complete combustion of the sample. During the "burn", a gentle oxygen stream is directed at the sample and the products of combustion are carried with this stream through the condenser, tritium exchange column and through the carbon-14 absorption column.

At the conclusion of the combustion, a superheated steam injection cleans the combustion chamber and connecting lines to the condenser.

A second injection of water into the condenser assures high tritium recovery. During the post combustion cycle, a nitrogen flush is used to eliminate explosion or fire hazards in the system and minimizes the quenching effect of oxygen in the system. The water collected is held in a glass vessel during the combustion and flush cycles. This then opens to the mixing chamber, and the tritium exchange column is rinsed three times with a nongelling scintillator, quantitatively transferring all the tritiated water into the mixing chamber. A final water rinse through the combustion chamber and primary condenser assures low carry-over and concludes the tritium combustion cycle.

In the carbon-14 or double-labelled combustion cycle, the gas stream continues from the glass holding vessel into the CO_2 absorber column, which prior to ignition is filled with an organic amine. The oxygen stream bubbles through the amine; the CO_2 reacts to form a soluble carbamate. The liquid column in the absorber is supported by the gas stream and, at the end of the combustion cycle, is expelled into the appropriate mixing chamber. The absorber column is washed three times with the scintillator mixture to assure quantitative transfer into the mixing chamber.

Once the combustion cycle is completed and the oxidizer portion of the OXI-FLO is clean, the mixing chamber is isolated from the oxidizer. A gentle stream of nitrogen bubbles introduced at the lowest part of the mixing chamber assures complete mixing of the liquids. After a five-second mixing, the contents of either mixing chamber are transferred with nitrogen pressure into the LSC counting chamber. Since quantitative transfer of the liquid is not practical (as learned by the designer's experiences with the RSP-B400 Processor) only a portion of the resulting sample is counted. Part of the sample is actually used to clean the counting chamber of the previous sample, then collected in a waste container.

Once the sample is in the counting chamber, sample counting begins simultaneously with a quick wash of the mixing chamber. Thirty seconds after the completion of the combustion cycle the OXI-FLO counts the sample and is ready for the next combustion cycle.

If double-labelled samples are processed or the counting time selected is longer than the combustion cycle time, the samples are held in the mixing chamber until the next combustion cycle can be initiated.

Admittedly, in the present configuration, samples labelled with both tritium and carbon-14 take twice the counting time, thus making the process too slow. In the commercial product, an optional second counting chamber will be available for the simultaneous counting of both isotopes, or to increase single sample throughput time.

Plans for the total automation of the system, that is, the addition of an automatic sample feeder on the front end of the instrument are under way. The aim is to produce an automated oxidizer/counter combination that will reliably process biological samples with accuracy, reliability and at a cost competitive with the presently available combined systems. Savings of considerable magnitude will be achieved in chemical use, labor and the elimination of vials.

EXPERIMENTAL

Several classes of radioactivity labelled, biological materials were used in the experimental OXI-FLO unit. All samples were

homogenized, and control samples were either solubilized with Solusol (National Diagnostics) or combusted in an OXI-ONE Sample Oxidizer and counted in both a Nuclear Chicago Mark II LSC or in a Radiomatic Instruments' RSP-B400 Sample Processor.

Table 1.

Test Substance	Activity	Solubilize		OXI-ONE		OXI-FLO	
		Recovery (%)	CV (%)	Recovery (%)	CV (%)	Recovery (%)	CV (%)
Liver	7500 dpm/g ^3H	83.5	5.82	98.9	1.23	99.8	3.29
Liver	28500 dpm/g ^{14}C	92.2	4.96	99.5	1.62	99.5	3.05
Bone Marrow*	14000 dpm/g ^{14}C	91.5	4.72	98.1	1.92	98.5	2.50
Muscle	12500 dpm/g ^{14}C	93.7	4.80	99.5	1.15	98.9	2.76
Blood	6000 dpm/ml ^3H	89.2	5.51	98.9	1.27	99.1	2.47
Blood	32500 dpm/ml ^3H	87.6	5.36	99.0	1.33	98.7	3.01

* Average 0.15 g bone marrow as processed.

All other samples were in the 0.3 to 0.4 g range.

A tabulation of results is given on Table 1. The coefficient of variation for the combusted samples were considerably better than that of the solubilized samples. The reason for that is the solubilization technique requires the heating of the samples during which, apparently, some loss of activity is also encountered.

While recovery figures for the OXI-ONE and OXI-FLO methods are compatible, the variance of the results from the OXI-FLO method is higher. This is attributed to the fact that while samples from the OXI-ONE were generally in the 15 to 18 mL range, the OXI-FLO only uses a fraction of this amount for the counting, therefore, the statistics of the counting are somewhat worse.

The sample-to-sample contamination (memory) of the OXI-FLO was determined by processing, in sequence, filter paper disks saturated

with tritiated and nonradioactive water. Each sample contained 0.2 mL water on approximately 0.1 g of paper. Table 2 is a compilation of the results. The memory of the experimental unit is somewhat higher than expected due to parts in the system which are machined instead of molded. The production systems are expected to exhibit a memory of about one-half that of the experimental unit.

Table 2

Sample No.	Sample	CPM (^3H)	Sample No.	Sample	CPM (^3H)
1	Blank	37	9	H_2O	610
2	T_2O	5972	10	H_2O	39
3	H_2O	105	11	T_2O	64976
4	H_2O	35	12	H_2O	580
5	T_2O	6012	13	H_2O	36
6	H_2O	92	14	T_2O	65116
7	H_2O	40	15	H_2O	600
8	T_2O	65205	16	H_2O	42

CONCLUSION

A system to prepare uniform low-quenching samples by oxidation and the immediate liquid scintillation counting of the samples was described. The advantages of the OXI-FLO system are faster turnover, savings in material and labor, as well as the possibility of complete automation of the radioactive measurement of biological samples. Preliminary experimental data indicates excellent reproducibility of a variety of samples.

REFERENCES

1. W. Schoniger, "Flask Combustion Method for the Determination of ^3H and ^{14}C Content in Biological Materials", *Mikrochim. Act.* 1955, 23.
2. R.T. Kelly, E.A. Peets, S. Gordon and D.A. Buyske, "Determination of ^3H and ^{14}C in Biological Samples by Rapid Combustion Techniques", *Anal. Biochem.* 2, 267, 1961.
3. V.T. Oliverio, C. Denham and J.D. Davidson, "Oxygen Flask, Combustion and Determination of ^{14}C and ^3H in Biological Material", *Anal. Biochem.*, 4, 188, 1962.
4. J.D. Davidson, V.T. Oliverio and J.I. Peterson in "The Current Status of Liquid Scintillation Counting", E.D. Bransome Jr. (ed.), p. 222, Grune & Stratton, New York, 1970.
5. J.I. Peterson, F. Wagner, S. Siegel and W. Nixon, "Design and Testing of Some Automatic Sample Introduction Valves", *Anal. Biochem.*, 31, 189, 1969.
6. J.I. Peterson, "Carbon Dioxide Collection Accessory of the Rapid Combustion Apparatus for Preparation of Biological Samples for Liquid Scintillation Analysis", *Anal. Biochem.*, 31, 204, 1969.
7. T.R. Tyler, A. Reich and C. Rosenblum in "Organic Scintillators and Liquid Scintillation Counting", D.L. Horrocks and C.T. Peng (eds.), p. 869, Academic Press, New York, 1971.
8. E. Rapkin and A. Reich, "Automatic Combustion for Routine Liquid Scintillation Sample Preparation", *Am. Lab.* 4, No. 10, 35, 1972.
9. D.W. Sher, N. Kaartinen, L.J. Everett and V. Justes in "Organic Scintillators and Liquid Scintillation Counting", D.L. Horrocks and C.T. Peng, (eds.), p. 849, Academic Press, New York, 1971.
10. J.E. Noakes and D.E. Taylor, "Spark Combusting Technique for Liquid Scintillation Sample Preparation", *Am. Lab.* 67-71, July 1976.