

SAMPLE OXIDATION FOR LIQUID SCINTILLATION COUNTING: A REVIEW

Walter E. Kisielecki and Evelyn M. Buess

Division of Biological and Medical Research
Argonne National Laboratory
Argonne, Illinois 60439, U.S.A.

ABSTRACT

The liquid scintillation spectrometer is a versatile instrument for the measurement and analysis of low-energy beta emitters, especially hydrogen-3 (tritium) and carbon-14. On the other hand, biological materials as well as environmental samples are most difficult to prepare as true solutions for liquid scintillation counting and present unique problems in sample preparation. To overcome problems of sample solubility, quenching, and chemiluminescence, a more universal preparation technique can be achieved if the sample is burned at red heat in an atmosphere of oxygen and the carbon and hydrogen converted into carbon dioxide and water and quantitatively dissolved in a scintillator to produce an unquenched sample. A number of methods of oxidizing samples for liquid scintillation counting are discussed. Experimental studies using carbon-14 and tritium are presented and potential application to biological and environmental problems are considered.

INTRODUCTION

The number and variety of toxic substances released into the environment as a result of modern technology increases year by year. Biological and medical research is aimed at the solution of the health-related environmental problems in order to develop understanding of the interrelationships between chemical and physical factors in the environment and human diseases. Since all organic compounds contain carbon and most contain hydrogen, the determination of carbon and hydrogen is the most frequently performed analysis. The liquid scintillation spectrometer is a versatile instrument for the measurement and analysis of low-energy beta emitters, especially hydrogen-3 (tritium) and carbon-14. However, biological materials as well as environmental samples are most difficult to prepare as true solutions for liquid scintillation counting and present unique problems in sample preparation.

The problems of sample solubility, quenching, and chemiluminescence can be overcome in a simple preparation technique if the sample is burned at red heat in an atmosphere of oxygen and then the carbon and hydrogen converted into carbon dioxide and water, respectively, and quantitatively dissolved in a scintillator to produce an unquenched sample.

In what follows, we shall review the general features of biological and medical investigations that place demands upon the design specifications of liquid scintillation counters and associated methodology. Special emphasis will be given to the oxidative technique for sample preparation. No attempt is made to review completely all biomedical applications, and most of the examples given are those originating in our own laboratory.

Biological variability places arduous demands upon a tool applied to biological problems. Individual members of an apparently homogeneous population of living systems do not all respond alike to the same stress or treatment, and the limits of normal response are usually quite wide in comparison with physical measurements. Such variability usually necessitates statistical interpretation of results, which calls for large numbers of determinations. In turn, large numbers of determinations require that the method of measurement be simple and involve as little sample preparation as possible. Simplicity and speed are often more to be desired than very high precision and accuracy, since biological variations are frequently the limiting factors in interpretation of results (1-3).

Also of importance is the wide variation in nature and composition of biological samples. Biological samples may be organic or inorganic. They may consist of chemically pure substances such as amino acids, steroids, and sugars, or complex mixtures such as animal excreta, whole organs and tissues, or suspensions of cells. Their solubility in the usual nonpolar scintillation solvents may vary from complete miscibility to essentially complete insolubility. Samples also may be completely inert to the scintillation process or they may be highly effective quenchers (4,5).

Biological investigations also require wide variations in sample size. Studies of natural levels of radioactivity or of contamination from worldwide radioactive fallout may require unusually large samples to supplement the sensitivity of detection. At the other extreme, the amount of sample

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available may necessitate measurements on extremely small samples. Examples of the latter are experienced in equilibration studies between the blood stream and the cerebral spinal fluid, or kinetics studies of the anterior chamber of the eye (6-8).

In summary, biological and medical investigations by nature call for counting systems with the greatest of versatility. Among the requirements are (a) analyses of large numbers of samples with a minimum of processing; (b) high sensitivity; (c) wide adaptability as to variations in sample size; and (d) accommodation of wide variations in nature and chemical composition of the sample. This, of course, is a difficult order for a single counting system to achieve, but liquid scintillation systems have shown considerable promise in being able to meet this challenge.

MATERIALS, METHODS, AND DISCUSSION

Liquid scintillation counting is now undoubtedly the most extensively used method for measuring weak beta-emitting isotopes, and various techniques have been developed for their measurement in the presence of biological tissues (9,10). The simplest of these techniques involves suspending or dissolving the tissue in a suitable scintillation medium. The severe quenching inherent in such methods, caused by the natural color of most biological materials, can be partially overcome by decolorizing with hydrogen peroxide. Despite this modification, however, the weight and nature of tissues that can be assayed by direct dissolution methods are limited.

Tissue, plasma, or urine can be also solubilized in aqueous or alcoholic solutions of alkali, or by quaternary ammonium hydroxide compounds and the resulting digest can be assayed. In samples prepared from such digest, however, color quenching is present to a degree that is dependent upon the heme or pigment concentration in the digested material, temperature and duration of heating during solubilization, and amount of digest material dispensed into each vial. Color may be reduced by an additional step, treatment with 30% hydrogen peroxide at the completion of digestion, or correction for quenching differences between samples may be made by preparation of samples with internal standardization or by other means. Unless the protein content is kept low, alkaline digests manifest phosphorescence which must be permitted to decay or be eliminated by the additional step of acidification before measurement (11,12).

A more universally applicable preparation technique for handling diverse biological materials is combustion and oxidation, leading to collection of tritium as water and carbon-14 as carbon dioxide. Several approaches, including wet and dry oxidation, have been developed to achieve this objective. Of these the most widely used procedure involves combustion in an oxygen-filled flask.

Oxygen-flask combustion of organic materials was first conceived as an analytical tool in 1892 by Hempel (13), when he combusted sulfur-containing coals suspended in a Pt basket within an oxygen-filled 10-liter bottle. Sixty years later, Mikl and Pech (14) discovered that oxygen-flask combustion was possible for routine semi-microanalytical analysis of sulfur and chlorine. It was a few years later that Schöniger (15,16) improved and extended the method to general analytical usage for various elements. The technique was first used in 1957 for radioactive measurement of carbon-14 by Gotte et al (17) and since then the method and its many variations have been most widely used (18-22).

The major disadvantage of the various oxygen-flask methods is that the combustion vessel is also the collection vessel for the gaseous products of combustion. Samples of scintillator containing absorbent for measurement must be removed from the collection vessel and thus contain variable amounts of quenching products, especially dissolved oxygen (23,24).

The severe quenching property of oxygen causes considerable attenuation and variation in the counting efficiency of the samples to be measured. Deoxygenation of the samples with nitrogen can be used to achieve equilibration of the sample, but stable counting rates are not achieved in all cases due to oxygen reabsorption. This reabsorption can be eliminated only under stringently controlled conditions.

A prerequisite of the oxygen-flask method for large samples is the need to dry biological materials before burning. If any of the tracer in the samples is present in a volatile form the use of the flask method in its simplest form, in which a number of samples are first dried in a vacuum desiccator and are subsequently burned in a combustion flask, is precluded. Such samples with volatile tracers must be processed individually. Existing oxygen train combustion procedures have been adapted by Dobbs (25) in a novel oxygen steam method that is applicable to freshly dissected tissues containing volatile tritiated compounds. In this method, oxygen

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at low pressure is passed through a radiofrequency field and is streamed over the undried tissue. Volatile components of the tissue are rapidly removed. At the same time, the oxygen ion reacts with organic material of the tissue leaving an inorganic residue. The oxidation products containing tritium are removed from the gas stream in a trap cooled with liquid nitrogen, and are subsequently dissolved in a liquid scintillator injected into the trap. The method could be a useful addition to the procedures in current use for assays of tritium in animal tissue because of the absence of memory effects, the absence of activity in the tissue residue, high trapping efficiency, and quantitative recovery of tritium from a maximum of 0.8 g of wet tissue. The method has not been adapted for isotopes other than tritium but does not lend itself to analysis of dual-labeled compounds. There is also a good deal of time and tedious labor associated with the method.

Standard vacuum-line combustion techniques have also been applied to similar problems with limited success; although these methods offer advantages in accommodating increased sample size, they are time-consuming and require elaborate equipment. They are also subject to serious cross contamination errors or "memory effects" due to highly absorbent oxidizing materials present in the system (26).

In our own laboratory a combustion apparatus was designed and constructed to combine the simplicity of the oxygen-flask method and the reproducibility and efficiency of vacuum-line techniques, including the convenience of a direct in-vial collection of the final products of combustion for direct liquid scintillation counting (27). This method has been referred to as the vacuum-line method.

Recovery values for carbon-14, sulfur-35, and tritium from different biological materials, with sample weights up to 250 mg of dried tissue material, average 98 percent and are reproducible and independent of the amount and nature of the material analyzed. The separation efficiency with double-labeled samples gives recovery values similar to those obtained when only a single isotope is measured.

In measuring double-labeled samples, only a single counting channel is necessary, since the isotopes are chemically separated. This means instrument settings and calibration procedures are not critical and can be optimized for each isotope. With these conditions, the range of ratios of one isotope to another that can be determined is greater than that possible by methods based on differences in energy spectra.

Continuing developments in combustion techniques have retained the oxygen combustion method, but have adopted classical microanalytical tube combustion methods, in which samples burn in a stream of oxygen. This more ideally meets the need for a method suitable for large-scale use (28-30).

The apparatus developed and designed by Peterson *et al* (31) evolved from earlier tube combustion methods. It collects tritiated water of combustion in cold solvent directly into a scintillation vial. At least 500 mg of dry sample weight can be accepted. The samples are contained in combustible capsules, allowing for a variety of sample types. By this method one investigator can prepare a sample ready for tritium counting every 3 minutes. According to the authors, the method has a collection recovery of 96%, is calibrated by internal standards, and shows a coefficient of variation of 2.0%. Peterson also described a carbon dioxide collection accessory for the previously reported tritium combustion system (32,33).

The utilization of vacuum-line techniques in combination with the basic Schöniger oxygen methodology has evolved into what many term the "second generation of oxygen combustion techniques." Dr. Niilo Kaartinen, at the University of Turku, Finland, developed an automated sample combustion technique incorporating these principles (34). His design has been commercially developed by the Packard Instrument Company of Downers Grove, Illinois. Recently, other manufacturers have produced similar apparatus (35-38).

With available instruments, it is possible to combust either tritium or carbon-14 samples, individually, or double-labeled samples containing both nuclides. The nuclides, in all cases, are collected in separate vials ready for counting. Separation of tritium and carbon-14 in this way allows optimum counting channels to be used in the spectrometer, therefore optimizing counting efficiency and eliminating tedious calculations correcting for carbon-14 spillover into the tritium channel (39-41).

These instruments provide recoveries of 97-98% for most biological samples with dry sample weights up to 500 mg; the precision of recovery has a standard deviation of $\pm 1\%$. From initial evaluation reports it appears that these instruments fulfill the many requirements to idealize combustion as a technique for sample preparation of biological materials for liquid scintillation counting (42-44).

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Biological, medical, and environmental studies are, to a large extent, concerned with how labeled compounds or molecules are metabolized, or incorporated into other substances or into cellular structures. The use of oxidative techniques to prepare samples for liquid scintillation counting for the detection and measurement of the low-energy beta emitters such as tritium and carbon-14 has extended the sensitivity of following biological processes by more than a thousand times over conventional analytical chemistry techniques.

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REFERENCES

1. C.H. Wang and D.L. Willis. Radiotracer Methodology in Biological Science. Englecliff, New Jersey : Prentice-Hall (1965).
2. W.R. Hendee. Radioactive Isotopes in Biological Research. New York : J. Wiley and Sons (1973).
3. W.E. Kisielecki in Liquid Scintillation, chap. 12. Fullerton, California : Beckman Instrument Company (1971).
4. Y. Kobayashi and D.V. Maudsley. Biological Applications of Liquid Scintillation Counting. New York : Academic Press (1974).
5. E.D. Bransome, Jr., Seminars in Nuclear Medicine 3, 389 (1973).
6. A.A. Moghissi, E.W. Bretthauer, E.L. Whittaker and D.N. McNelis, Int. J. Appl. Radiat. and Isot. 26, 339 (1975).
7. M.E. Hinkle, U. S. Geol. Surv. Prof. Pap. No. 750-B, B171 (1971).
8. V. Nuti and B. Bacci, Farmaco. Edizione Pratica. Pavia. 27, 381 (1972).
9. J.B. Birks. The Theory and Practice of Scintillation Counting. New York : Macmillan and Co. (1964).

10. E.F. Polic in Instrumentation in Nuclear Medicine, Vol. I, p. 181 (G.J. Hine, Ed.). New York : Academic Press (1967).
11. E. Rapkin in Instrumentation in Nuclear Medicine, Vol. I, p. 181 (G.J. Hine, Ed.). New York : Academic Press (1967).
12. P.E. Stanley, Atomic Energy in Australia 13, 29 (1970).
13. W. Hempel, Z. Angew. Chem. 1892, 393 (1892).
14. O. Mikl and J. Pech, Chem. Listy. 46, 382 (1952).
15. W. Schöniger, Mikrochim. Acta. 1955, 123 (1955).
16. W. Schöniger, Facts and Methods for Scientific Research 1, 1 (1960).
17. H. Gotte, R. Kretz and H. Baddenhauser, Angew. Chem. 69, 561 (1957).
18. W. Mertz, Mikrochim. Acta. 1959, 640 (1959).
19. A.R. Britt and W.E. Kisieleski in Abstract of Papers - 167th ACS Meeting. Abst. #41. Los Angeles (1974).
20. H.W. Knoche and R.M. Bell, Anal. Biochem. 12, 49 (1965).
21. G.N. Gupta, Microchem. J. 13, 4 (1968).
22. R.G. Kelly, E.A. Peets, S. Gordon and D.A. Buyske, Anal. Biochem. 2, 267 (1961).
23. W.D. Conway and A.J. Grace, Anal. Biochem. 9, 487 (1964).
24. L.M. Hunt and B.N. Gilbert, Int. J. Appl. Radia. and Isot. 23, 246 (1972).
25. H.E. Dobbs and G.M. Land in Int. Conf. Radioactive Isotop. Pharmacol., p. 121 (P.G. Waser, Ed.). London : Wiley-Interscience (1969).
26. D.A. Kalbhen in Sym. on Liquid Scintillation Counting, Vol. I, p. 149 (A. Dyer, Ed.). London : Heyden and Sons, Ltd. (1970).
27. L.G. Huebner and W.E. Kisieleski, Atompraxis 16, 1 (1970).

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28. B.S. McEwen, *Anal. Biochem.* 25, 172 (1968).
29. S. Mlinko, E. Fischer and J.F. Diehl, *Zeit. Anal. Chem.* 261, 203 (1972).
30. S. Von Schuching and C.W. Karickhoff, *Anal. Biochem.* 5, 93 (1964).
31. J.I. Peterson, F. Wagner, S. Siegel and W. Nixon, *Anal. Biochem.* 31, 189 (1969).
32. J.I. Peterson, *Anal. Biochem.* 31, 204 (1969).
33. T.R. Tyler, A.R. Reich and C. Rosenblum *in* *Organic Scintillators and Liquid Scintillation Counting*, p. 869 (D.L. Horrocks and C.T. Peng, Eds.). New York : Academic Press (1971).
34. N. Kaartinen *in* *Packard Technical Bulletin #18*. Downers Grove, Illinois (1969).
35. E. Rapkin and A. Reich, *Amer. Lab.* Oct, 35 (1972).
36. D.W. Sher, N. Kaartinen, L.J. Everett and V. Justes, Jr. *in* *Organic Scintillators and Liquid Scintillation Counting*, p. 849 (D.L. Horrocks and C.T. Peng, Eds.). New York : Academic Press (1971).
37. J.E. Noakes and W.E. Kisieleski *in* *Liquid Scintillation Counting Recent Developments*, p. 125 (P.E. Stanley and B.A. Scoggins, Eds.). New York : Academic Press (1974).
38. R.J. Harvey Instrument Corporation, *Advertising Literature*. Hillsdale, New Jersey (1975).
39. H.W. Hilton, N.S. Nomura and S.S. Kameda, *Anal. Biochem.* 49, 285 (1972).
40. T. Fujimori, T. Takesue and K. Ishikawa, *Chem. Abstr.* 79, 21370 (1973).
41. S. Baba, Y. Baba and T. Konishi, *Anal. Biochem.* 66, 243 (1975).
42. L.H. Scroggins, *J. Assoc. Off. Anal. Chem.* 56(4), 892 (1973).

43. R.A. Zaroda, Clin. Chem. 15, 555 (1969).
44. J.B. Ragland in The Nucleus #20. Des Plaines, Illinois : Nuclear Chicago Corp. (1966).