

Homogeneous Counting

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INTRODUCTION

The rapid and astonishing advances in the technique of liquid scintillation counting have been due in large measure to the instrument designers. In the last 20 years such counters have gone from a single tube in a refrigerator-cum-glove box to the completely automatic, highly stable, computer coupled systems in use today. The speed with which new developments in electronics hardware and software logic have been integrated into liquid scintillation counters has been most gratifying. It is common practice for a user to insert several hundred samples into a counter on Friday and pick up his data computed as d.p.m., weight %, volume %, titer, etc. on Monday morning. Why, then, do we still get some wrong answers?

The explanation seems to be to a large extent due to the sample preparation methods, particularly of organic chemical or biological origin. It is simply that the great convenience and reliability of the modern instruments has led to the application of the methods to a bewildering variety of samples, many of which were and are unsuitable for analyses without careful preparation. As in most analytical techniques, the instrument has outstripped the sample preparative methods. This is also obvious from the literature where the bulk of recent publications is concerned with improvements in sample preparation, and very few with improved instrument performance.

A plenary lecture is supposed to review the current state of a subject, thus setting the stage for the working papers which follow. I wish to change that approach somewhat, but not completely, by presenting a selected review of the literature, not necessarily current, interspersed with personal opinions about the strengths or weaknesses of a particular technique. This will become most apparent in the preparation of biological samples which account for the bulk of samples today, although as a petroleum chemist I have rarely had to deal with such samples. However, I have followed the general literature over the years with a sense of increasing uneasiness over a situation developing to the point where the use of solubilisers, digesting systems, etc. seems to have gained a firm place in the radioanalytical arsenal as evidenced by the large number of papers on this subject and the steady availability of new reagent systems by research workers and by commercial houses. I hope to present a rationale for

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the discontinuance of the use of such systems and a return to combustion as a sample preparative tool.

This chapter on homogeneous sample preparation will contain three sections — gases, inorganics and organics. Before I begin I suppose I should define a homogeneous sample (for liquid scintillation counting). This is a sample in which the composition of the volume traversed by the emitted radiation is, for all practical purposes, the same everywhere. Note that this, therefore, does not require true solution, i.e. homogeneity on the molecular level, but on a level considerably higher — perhaps $<0.1\mu$ radius for tritium. By this definition a cocktail homogeneous to one isotope may not be so to another because of different beta ray energies. In this sense true solution is a sufficient but not a necessary condition.

Considered in this way, gel counting with surfactants or cabosil is homogeneous if the droplet or particle sizes are small with respect to the beta particle energy. The reason for making this distinction is that homogeneous sample preparation does not restrict the user to true solutions (i.e. small molecular dimensions) but offers the option of using any techniques which disperses the sample in the counting system while avoiding absorption or adsorption losses.

GAS SAMPLES

This section is restricted to the analysis of gases based upon their solubility in the counting fluid rather than upon chemical reaction, as in CO_2 absorption by base.

Horrocks and Studier¹ in 1964 measured the solubility of some radioactive noble gases (^{133}mXe and ^{220}Rn) and found that if the counting vial had a small vapour space, the bulk of these gases would dissolve in the liquid. A factor expressing the ratio (cm^3 in liquid/ cm^3 in vapour space) was five for Xenon-131, 32 for Radon-222 and 0.9 for Krypton-85. The latter value was too low for quantitative analysis. Later Horrocks² applied this to CO_2 in toluene where the ratio 4.4 was found, albeit at -20°C . Gordon et al.³ applied the method to H_2^{35}S in a toluene scintillator and Curtis et al.⁴ applied it to tritium gas as well as Krypton-85. The method may be of interest for noble and other rather inert gases but it seems superfluous for reactive gases such as $^{14}\text{CO}_2$ which can be readily trapped in appropriate counting solutions containing bases like phenylethylamine. It is actively employed by us for counting hydrocarbon gases using a vial with a constricted neck with a small silicone rubber plug. The plug is vacuum tight and samples are injected by hypodermic syringe. It is the accepted method for the analysis of olefinic monomers used in polymerisation. Homopolymers made from these labelled olefins are also analysed and must have the same specific activity as the monomer before the work proceeds. Gaseous effluents from gas chromatography have also been trapped and counted. This is not strictly gas counting since compounds so analysed may be liquids or even solids at room temperature. On balance, gas analysis by liquid scintillation counting has only limited interest as indicated in the literature. The use of vacuum manifolds, sealed glass vials, etc. tends to limit its general popularity and I recommend the rubber plug plus hypodermic approach for most convenient operation. Calibrated hypodermics are used for highest accuracy and one can expect sampling errors less than 2% relative.

INORGANIC SAMPLES

Liquid scintillation cocktails are inherently inappropriate for ionic substances. However, the great convenience of this technique has led to considerable efforts in sample preparation to overcome the hostility of the usual scintillation solvents towards such materials. Three general approaches are: (1) solubility by use of

more polar solvents such as ethers or alcohols; (2) alteration of the sample, particularly metals, to an oil soluble form; (3) avoidance of wall adsorption losses or precipitation by providing a very large surface for adsorption, viz. cabosil.

Carr and Parson⁵ measured Calcium-45 as the chloride in a cocktail having 30% ethanol, Fairman and Sedlet⁶ determined Lead-210 by conversion to the nitrate and solution in a dioxane scintillator. Methods to analyse both radioisotopes of iron (⁵⁵Fe and ⁵⁹Fe) by liquid scintillation counting have employed precipitation as the hydroxide followed by conversion to the chloride, reduction with ascorbic acid and direct solution. Separation of the two isotopes was readily achieved because of the weak 5.9 keV X-ray of Iron-55 and the strong 0.4–1.5 MeV betas of Iron-59.

In this regard it is often the belief that the development of an aqueous compatible cocktail (e.g. Bray's solution, dioxane, ethanol/toluene) is adequate for aqueous solutions of salts. This is certainly a dangerous assumption since the behaviour of the salt may be quite different from that of the water. To avoid this error Bush⁷ devised a double ratio technique for determining whether one was counting in true solution. The method involves plotting the external standard two channel ratio versus the sample two channel ratio. Departure from a standard curve previously developed is indication of adsorption or precipitation of the sample. The method has been successfully applied by Glass⁸ to polymers and by Laurecot and Hempstead.⁹ In addition, transfer of the sample to a fresh counting vial will indicate whether any activity is fixed to the walls of the vial, according to Bush.⁷ However, it might not indicate the formation of a fine or flocculant precipitate which is also transferred. This useful technique really applies to homogeneous counting of which solution counting is one example. We have applied the Bush⁷ technique to polyolefin counting and found that an obvious gel also fits the solution curve.

Conversion of inorganics to a more soluble, non-ionic form is a more desirable and analytically safer method of preparation. Nuclear fuel analysts have used the alkylphosphates of the heavy metals as the solutes. Elements such as promethium,¹⁰ zirconium¹¹ and plutonium¹² are examples of organophosphate complexes. Other organic reagents include 2-ethyl-hexanoic acid for cadmium, lead, bismuth,¹³ and caproic acid for nickel.¹⁴

Using the principle of 'if you can't beat them, join them', Blanchard and Takahashi¹⁶ added cabosil to a toluene/ethanol counting solution to provide a very large surface for adsorption of labelled molecules. He showed that for an inorganic ($\text{Na}_2^{35}\text{SO}_3$) and several Carbon-14-labelled polymers a dramatic increase in the stability of count rate was achieved by a 3% w/v concentration of the silica. One would expect that this technique would show homogeneous counting by the Bush double ratio method. More energetic isotopes (e.g. Chlorine-36, Phosphorus-32) should certainly show such a response.

In all of the solution approaches to counting inorganics, whether salts or complexes, the concentration of the sample is of great importance. This is because the vial wall has a definite capacity for the labelled sample. For example, 20 ml of sample in a normal glass counting vial exposes about $3 \times 10^{17} \text{Å}^2$ of glass surface. Assuming no roughness factor and a simple value of 5Å^2 for the ionic area, we find that the glass has a capacity of 6×10^{16} molecules or 10^{-4} mmoles. For calcium chloride this corresponds to about 0.01 mg or a concentration of about 0.5 p.p.m. Thus a concentration of a few p.p.m. of a salt may result in a significant error in analysis. This in fact is not unique to inorganic materials. Hayes¹⁷ in 1953 reported that 0.1 p.p.m. of benzoic acid in a toluene scintillator produced a counting efficiency of half that when 2 p.p.m. and higher concentrations were used. Eicholtz

et al.¹⁸ studied the adsorption of radioisotopes on glass and plastic surfaces from aqueous solutions. Not only glass adsorbed these materials (cations) but, surprisingly, polyethylene, polypropylene and silicone coated glass as well, but to varying degrees. The general conclusion was that the addition of carrier was the most effective means of reducing the adsorption. This, of course, suggests that carrier-free concentrations (usually in the p.p.b. region) of nuclides must be avoided. The problem then arises as to how much carrier to add since the problem of incipient precipitation always exists albeit more for ionic materials than organic complexes. The use of the Bush⁷ double ratio method is certainly indicated in these cases to keep one out of serious trouble in the analysis. One must also bear in mind that some precipitation and/or adsorption reactions may appear hours later so that data on count rate versus time is essential to establish that not only the short-term but the long-term condition of the sample is stable and reproducible. This is best done by the use of standards having the same molecular structure as the sample. Standardisation of inorganic samples may be done by any of the accepted techniques, i.e. internal standard, external standard or sample channel ratio. Use of a standard labelled cation or anion in another chemical form from the sample is hazardous unless one clearly establishes that both have exactly the same distribution during the counting period.

ORGANIC SAMPLES

This section will deal with those samples which by the nature of their physical or chemical properties are unsuitable for direct homogeneous liquid scintillation counting. Those types which are readily soluble in the various liquid scintillation solvents, such as alcohols, hydrocarbons, ethers, carbonyls, esters, carboxylic acids, etc. need little further comment. The principle of carrier dilution should, however, be applied even to these samples of high polarity and low concentration simply because adsorption is always a threat. In addition, the permeability of polyethylene to aliphatic molecules (i.e. alkanes) is well known and one should exercise care when storing or counting such samples for long periods. Dioxane scintillator or Bray's cocktail are particularly appropriate solvents for those materials which are water soluble, highly polar, or both.

Samples of intense colour, low solubility or persistent chemiluminescence (e.g. recycle oil from refinery catalytic cracker) must be converted to an amenable form and combustion seems the only certain technique. In biological work, unlike petroleum or petrochemical studies, the use of an alternative route, i.e. digestion and/or solubilisation, has gained wide acceptance and I wish to deal with this technique before discussing the various combustion techniques now available.

The function of the solubiliser, whether it be acidic or basic, is to reduce a sample composed of high molecular weight compounds to a variety of fragments which by action of the powerful solvent ability of the reagent are then prevented from precipitation or agglomeration. Neither the chemical composition of the fragments nor their molecular weight distribution are known. It is sufficient that a visually clear solution is achieved which, when added to an appropriate scintillator cocktail, does not change in appearance or count rate with time. In the last 5–10 years a sizeable literature has grown around the use of such sample preparation treatments, the reason being that the large numbers of samples to be processed discouraged the use of the then existing combustion methods. However, for reasons advanced below, this procedure is fraught with danger and should be reconsidered as a method of analysis.

In 1958–59 we began studying the adsorption of various molecules at the oil/metal interface. At that time we came across the work of Binford and Gessler¹⁹ who were studying the adsorption of different polyisobutylene polymers on carbon

black from organic solvents. The polymers were of various molecular weights ranging from 5000 to over 300,000 and of low polarity having good solubility in hexane. Their studies demonstrated that above a certain molecular weight adsorption from hexane onto the carbon black was irreversible and was not dependent on unsaturation but only on molecular weight. Smith et al.,²⁰ studying the adsorption of an oleophilic detergent calcium dinonylnaphthalenesulphonate Sulphur-35 at the gold/oil interface, found that the detergent was displaced from the gold surface by a polymeric alcohol (MW=20,000) or a polydodecyl methacrylate (MW=50,000). Further work using Carbon-14-labelled polymers showed that adsorption at the iron/oil interface was irreversible for polyalcohols, polyamine and polythiol of MW's from 20,000 to 500,000. Attempts to displace the adsorbed polymers with polar solvents or even pure monomer were unsuccessful. Increasing the temperature to 200°C for several days served only to remove some 20% of the amount adsorbed. Blanchard and Takahashi¹⁶ reported the striking fact that acrylamide Carbon-14 in counting solution did not change in count rate with time but polyacrylamide Carbon-14 decreased. Glass⁸ reported that an homogeneous-appearing polymer solution was shown to be non-homogeneous by the double ratio method.

The rationale for such behaviour of polymers may be advanced as follows: a polymer in a semi-coiled or uncoiled state in solution encounters a solid surface on which some of the repeat monomer units adsorb, albeit weakly. If the polymer then uncoils onto the surface, as observed by Binford and Gessler,¹⁹ all or most of the adsorbing groups will be in a dynamic equilibrium between adsorption and desorption, i.e. some will be adsorbing and some desorbing, not, however, all at the same time. Assuming the heat of adsorption is low, 1-2 kcal/monomer unit, and that 500-1000 of the functional groups are exposed at any one time, it is clear that the probability that all of the adsorbing groups are off at the same time is exceedingly small. Stated another way, it would take several hundred kilocalories to desorb all the groups simultaneously, a requirement greater than C-C bond strength. It is also clear that as long as even one functional group of the polymer is fastened to the surface, the entire polymer is held close to the surface and counting geometry will be less than 4π . If, in the case of a non-polar polymer,¹⁹ adsorption becomes irreversible at some molecular size (~50,000), it clearly becomes irreversible with polar polymers at much lower molecular weights. This is the situation prevailing for the highly polar macromolecules originating from biological studies. Furthermore, if one bears in mind that the behaviour of such polymers is profoundly influenced by the nature of the solvent, the prior history of the polymer treatment, as well as the solid surface, one can see that predictability of surface behaviour is virtually impossible and analysis becomes uncertain. The quantitative effect arises from the calculations made for the capacity of the vial wall for inorganic ions. If, for example, a rather heavily coiled polymer of MW 10^5 has only a few groups of about 25\AA^2 - 100\AA^2 cross section anchored to the surface, it is obvious that as much as a milligramme may be adsorbed on the glass wall from a counting solution. This means that sample sizes several times this order must be used to minimise the effect of these losses.

Because of macromolecule adsorption behaviour and the impossibility of correcting for it, it is my conviction that samples of this nature should always be burned and the products collected for counting. In our laboratory all polymers of the polyolefin type are burned, polymers of the polyaromatic type (polystyrene) are dissolved in amounts never less than 100 mg and usually 500 mg in 20 ml of a toluene scintillator. Such analyses are routinely checked by combustion (1 in 10) to make sure we are not fooling ourselves.

One is not out of the wood even if total degradation of proteins to amino acids is

achieved. Thus Litt and Carter²¹ showed serious adsorption losses of simple amino acids such as arginine and lysine depending upon the cocktail used and the state of the vial surface. For slightly more complex molecules labelled with Carbon-14, the effect of the vial surface varied with the nature of the compound. In one case plastic was superior to silanised glass; in another case they were similar. Generally, plastic vials were better, as expected.

Some workers have expended considerable effort to develop a general arsenal of methods to process the incredible variety of samples requiring liquid scintillation counting. Grower and Bransome²² counted polyacrylamide gels following electrophoresis of tritium-labelled gel sections. They found that special slicing of the gel followed by hydrolysis of the gel was needed to obtain results within a few percent of the combustion value. Bray²³ studied a series of preparative methods for radioassay of aqueous samples. He screened several solubilisers as well as various cocktails and different kinds of samples. One must conclude from his careful study that each new sample type as well as each new labelled compound must be carefully checked under a variety of conditions to ensure that the final method used will yield accurate data. Kahlben and Rezvanie²⁴ described work involving seventeen scintillation cocktails, three solubilisers and two methods of oxidation. It was clearly demonstrated that with each system different problems were raised. These included chemiluminescence, varying efficiencies with sample volume, phase separation, etc. Little and Neary²⁵ showed that for each sample type and solvent an optimum fluor concentration exists. In other words, there is an optimum cocktail for each sample — a major problem for the user who wishes to operate under optimum conditions but has a variety of samples, some of virtually unknown composition. Kahlben and Rezvanie²⁴ concluded that it would be greatly to the benefit of workers in this field if only a limited number of sample preparation methods were used, viz. combustion or oxidation for solids and emulsion cocktails for liquids.

The situation, then, that we have got ourselves into is the development of a large variety of scintillation cocktails and a number of hydrolysing reagents to cope with an almost infinite variety of samples. In the effort to speed up sample preparation methods we have inadvertently introduced a number of other problems which need watching. Kahlben and Rezvanie²⁴ are indeed correct in calling for a simplification so that techniques and results can again become comparable and the user is assured of the accuracy (as opposed to the precision) of the analyses. In addition, the macromolecular adsorption problem described above raises the very real possibility that during an experimental study a small change in molecular weight, in molecular structure, or degree of unfolding of a polymeric chain can invalidate a sample preparation method carefully developed for the type of samples expected. I find it hard to believe that there is any programme using liquid scintillation counting which prefers a large amount of uncertain data to a smaller amount of accurate data. For organic samples of indeterminate composition the only way to ensure accuracy is by combustion.

COMBUSTION METHODS

Combustion of labelled organic samples preparatory to radioassay was in use years before liquid scintillation counting. Samples were burned then either in high pressure oxygen bombs, in combustion trains or in curius tubes with concentrated nitric acid. Carbon-14 was normally assayed as barium carbonate and Sulphur-35 as barium sulphate on planchets with thin window or windowless proportional counters. Tritium was assayed by converting the water from combustion to hydrogen gas over iron, zinc or magnesium and was introduced into a geiger or proportional gas counter or ion chamber for measurement.

These were tedious methods requiring considerable manipulative skill. The advent of liquid scintillation counting came as a great relief to those of us using the above techniques. Since then sample combustion methods have undergone radical changes in order to adapt them to the liquid systems found to be appropriate for scintillation counting.

Wet combustion

Samples may be wet or dry combusted. Wet combustion developed years ago in the petroleum industry, for trace metal analysis employs strong acids. The sample is totally destroyed and one must then somehow collect the $^{14}\text{CO}_2$ evolved. It is inappropriate for tritiated biological samples unless one condenses the acid and water vapour. Wet combustion as practiced by Mahin and Lofberg^{26,27} is in fact not combustion in the conventional sense but a more drastic treatment of the biological samples than the alkaline solubilisers currently in vogue. They employed 60% perchloric acid/30% hydrogen peroxide at moderate temperatures to degrade the macromolecules to smaller, soluble molecules. A scintillation cocktail compatible with these acids (ethylene glycol monomethyl ether plus toluene) was added. The cocktail must be carefully chosen because of quenching and chemiluminescence problems with some systems (Bray's solution, gel systems). Wheeler and Strother²⁸ compared the NCS solubiliser with van Slyke oxidation to CO_2 and absorption in ethanalamine. It was found that ring-labelled compounds were not attacked. Van Slyke oxidation with hot chromic acid may be adequate for biological samples, may not be for many organic chemical types, and certainly is not for petroleum-based samples. Bartley and Abraham²⁹ oxidised paper chromatograms with potassium persulphate and absorbed the evolved CO_2 after acidification. It was effective for sugars and organic acids, including amino acids. However, earlier work on petroleum-based samples showed that persulphate was also ineffective for ring destruction, particularly cata-condensed aromatics (anthracene and higher). Smith³⁰ analysed organic samples for Chlorine-36 by total destruction with $\text{H}_2\text{SO}_4/\text{HNO}_3$ and distillation of the HCl into caustic. Counting was in a dioxane-based scintillator after neutralisation and buffering.

On balance it seems that wet combustion of organic samples can be an effective method if total decomposition is achieved. Anything less involves many of the uncertainties found with alkaline solubilisation. For Carbon-14 it may involve considerable manipulation and it is generally not employed for tritium. When used for non-volatile or easily isolated radionuclides (metals, halides),²⁷ total destruction of the sample can be successfully employed. The uncertainties of adsorption or precipitation must, however, be avoided through the judicious use of carriers.

It is clear from the foregoing that the virtue of complete destruction of a sample lies not only in the advantage of being able to count the sample homogeneously but, more important, in the conversion of the radionuclides into known chemical forms for which appropriate counting methods are available. On this basis the dry combustion methods hold the most promise, particularly for Carbon-14, tritium, and Sulphur-35.

Dry combustion

There are several methods of dry combustion of organic samples which have been reported in the literature. These may be divided into sealed systems and flowing systems. The former include high pressure bombs and atmospheric pressure oxygen flasks; the latter include combustion train systems and, recently, a flowing flask system.

The Parr oxygen bomb is an example of a sealed system. It comes in various sizes and operates with 20–25 atm oxygen. The sample is held in a platinum cup and ignited via an electrically heated filament. Acidic gases are trapped by placing several milligrammes of an aqueous caustic solution in the bottom of the bomb. Sample size capacity of a 500 ml bomb is about 1 g. Water of combustion can also be trapped by chilling the bomb before venting. The method is quantitative. Sheppard and Rodegher³¹ used the system for Carbon-14 and tritium. The limitation is the high cost of a bomb and the rather long manipulation involved in setting up, venting and clean-up for each sample. Scott and Kennally³² used much less costly sealed Vycor tubes containing oxygen slightly below atmospheric pressure. Samples ranging in size from 6–100 mg of carbon could be combusted in various size tubes by heating to 850°C for about 5 min. The tubes were chilled, opened and activity removed with either a dioxane or toluene/amine cocktail depending on whether water or carbon dioxide was collected. Yields were high, 96–98%, and the method well suited to large numbers of samples. One drawback was the need for glass manipulation and sealing. Explosion hazards are also present. Some 20 samples could be processed per day.

The Schoniger flask method for the analysis of various elements in organic samples lends itself admirably to the radioassay of Carbon-14 and tritium. The various modifications described in the literature include electrical ignition, infrared ignition, placement of absorbing scintillator in a cold finger before ignition or injection after ignition, and the use of simple side arm flasks with rubber stoppers as well as more complex three-neck flasks. In addition, combustion directly in a counting vial has been reported as a method for mass production.

Thus Oliviero et al.,³³ using a 2-litre filter flask and infrared ignition, injected, following combustion, a phenylethylamine-containing scintillator via rubber tubing attached to the side arm. Dobbs³⁴ overcame the quenching presence of halogen in the sample by adding pentene-2 to the absorbing scintillator solution injected into the flask after ignition. Conway et al.³⁵ demonstrated near quantitative recovery for $^{14}\text{CO}_2$ (97%) and $^3\text{H}_2\text{O}$ (98%) when using ethanol-in-toluene to absorb the water and ethanolamine plus cellosolve-in-toluene to absorb the CO_2 . Gupta³⁶ described a novel technique for in-vial combustion of biological samples (2–3 mg) which is well suited for large numbers of samples. Vickers³⁷ improved the Gupta method by providing more certain ignition, larger (6 mg) samples and elimination of leaks. Ronucci et al.³⁸ determined simultaneously Hydrogen-3 and Sulphur-35 in samples by oxygen flask combustion and trapping the sulphur oxides and $^3\text{H}_2\text{O}$ in methanol/dioxane cocktail containing phenylethylamine.

Sample size limitations of the oxygen flask (usually ~100 mg sample plus 100 mg paper) were overcome by Dobbs³⁹ by the use of 5-litre flasks for samples of 2–3 g size, dry weight. He also extended the method to organic samples on inorganic substrates by adding powdered cellulose to ensure complete combustion. A significant advance in the oxygen flask method has been reported by Sher et al.⁴⁰ where sample combustion is aided by a stream of added oxygen and the combustion products are swept continuously into appropriate absorbers. Water adsorption effects are minimised by a steam purge. The method has been applied to Carbon-14 and Hydrogen-3 radioassay and seems to go a long way towards the automation of the combustion step of a sample preparation. Several studies of the performance of this unit have been reported^{40,41} which demonstrate its quantitative nature and the absence of a tritium memory effect. Hunt and Gilbert⁴² compared the combustion method with alkaline digestion on a variety of biological samples. They found lower recoveries in all cases with the digestion step.

The oxygen flask method is one of the most useful for combustion of organic

samples. It lends itself well to scale-up to large numbers as well as large amounts of sample. It is suitable for virtually all radioisotopes. The major limitation seems to be the large amount of glassware clean-up and decontamination necessary for a high sample throughput. In this regard the Kaartinen—Packard⁴⁰ flow combustion flask is a significant step towards more efficient operation. A problem reported for the oxygen flask as well as other combustion methods involves the variable quenching by oxygen present in the counting solution.⁴³⁻⁴⁵ Following combustion the counting solution contains a considerable amount of oxygen which, on standing in the counter, may slowly equilibrate with air (rapidly if opened for introduction of an internal standard). This increase of efficiency with time may lead to erratic results. A few minutes air purge avoids the problem. A nitrogen purge should not be used because of a similar effect but in the opposite direction. The use of the external standard method to obtain the counting efficiency of a sample saturated with oxygen is not recommended because it determines counting efficiency at a time different from the sample count and may lead to errors. Furthermore, a drifting count rate may conceal an instrumental or sample instability problem. Stable counting rates should be present, and an air purge avoids the variable oxygen effect. The advent of air-tight counting vials at low cost would, of course, change this.

Combustion train

Combustion tube analysis of organic samples for Carbon and Hydrogen is an old and highly developed procedure. Very high accuracy for Carbon and Hydrogen is achieved with such systems which employ hot ($\sim 800^{\circ}\text{C}$) CuO to complete the combustion of samples heated in a stream of oxygen. Various additional packings in the train are sometimes used to eliminate nitrogen oxides, halogens and sulphur. The procedure is well suited to the preparation of samples for liquid scintillation counting since it simply entails placing a trap on the exit end of the combustion tube to collect the $^{14}\text{CO}_2$, $^{35}\text{SO}_2$ or $^3\text{H}_2\text{O}$. It is not appropriate for non-volatile radioisotopes, and has been most often employed on samples in the milligramme range.

However, Peets et al.⁴⁶ used a triple combustion tube set-up to burn three samples at a time, up to 2 g each, and could process 24–30 samples a day, collecting the tritiated water in cold traps. Freeland⁴⁷ burned up to 6 g of carbon, collecting the CO_2 in caustic and liberating the CO_2 with acid to prepare methyl benzoate via a Grignard reaction. This method is largely of interest in age dating where activities are very low. Knoche⁴⁸ burned samples for tritium analysis in an argon/ O_2 mixture and trapped the water for subsequent analysis. Griffiths and Mallinson⁴⁹ used 100–300 mg samples to run up to 40 samples a day on a conventional C/H apparatus. They trapped the water in dry ice and the CO_2 in a succeeding trap containing phenylethylamine plus methanol (15 ml of 2:1). Smith⁵⁰ used a Leco apparatus based on rapid induction heating of a sample mixed with iron chips, CuO, H_2SiO_3 and MoO_3 . Combustion required 3 min for a 120 mg sample and an O_2 flow rate of 300 ml/min. Absorption was in an ethanamine-based scintillator, two traps in series. The second trap absorbed 1–3% of the $^{14}\text{CO}_2$ at ice temperature. Our experience with an automatic combustion train, i.e. where the combustion rate is controlled by pressure sensors, is that 20–40 min per sample are required for samples from 100–500 mg. Trapping is via a small tower with a sintered disc disperser and either a dioxane or a phenylethylamine cocktail to trap water or CO_2 at ice temperatures. Recovery is quantitative and the 1–2% tritium memory effect is eliminated by following every tritium sample with 150 mg of water. All samples are automatically air purged at the end of the combustion.

The major disadvantages of the combustion train approach have been the high cost of the equipment (compared to oxygen flasks), the long analysis time and the rather careful attention required to ensure a smooth combustion. This is not the case with the controlled combustion system mentioned above. However, the proven quantitative aspects of the combustion train indicated that it was ripe for some major improvements to raise its appeal to radiochemists. This appeared in the Peterson^{51,52} apparatus. This unit combines combustion, trapping and delivery into a liquid scintillation counting vial ready for capping and counting within 3 min. Samples up to 500 mg for Hydrogen-3 and slightly less for Carbon-14 are routinely handled. Samples are placed in Lexan or other capsules which are dropped into the combustion tube. Some studies made of the Petersen combustion train^{53,54} showed it to be a fast, convenient unit requiring some operator skill and yielding absolute recoveries of Carbon-14 and Hydrogen-3 of 93–96% and 97% respectively. Memory effects are in the 0.2–0.4% region. The apparatus is certainly expensive but its speed and convenience make it an interesting possibility for the heavily loaded laboratory. According to Petersen et al.⁵¹ the samples increase in counting efficiency with time. This is most likely due to slow equilibration with air and would be improved by including an air purge step. The consistently low recovery of Carbon-14 may be due to the dissociation of the carbamate. If so, keeping the absorbing cocktail cold will reduce this.

A final comment on methods of incorporating products of combustion into homogeneous counting systems. These involve chemical reactions to convert water or carbon dioxide into a low quenching organic liquid. Thus Moghissi and Kogube⁵⁵ reacted water with a benzyl Grignard to make toluene which could be distilled and counted. Only half of the tritium is recovered in this reaction but very large samples can be handled. Polack and Stipp⁵⁶ and Kearns⁵⁷ reacted CO₂ with lithium to make the carbide which was converted to ethylene and finally to benzene. This technique is of interest for large samples of low activity where the highest figure of merit — (sample size) x (efficiency) — is required, i.e. age dating, natural tritium concentrations, very high dilution experiments.

CONCLUSIONS

In conclusion, then, it seems that gases, inorganic samples and organic samples can be successfully analysed in homogeneous systems. One must be aware, however, that an homogeneous appearance may be desirable, but is not a sufficient, condition for accurate radioassay. It also may not be necessary if the β -energy is large compared to the particle size. Adsorption and deposition of the radionuclide must be ruled out by careful testing, such as the double ratio method of Bush,⁷ by transferring the counting solution to a fresh vial, or by comparison of the sample channel ratio with an internal standard channel ratio. One should avoid extremely low concentrations of a labelled sample because the adsorption capacity of the vial wall is high enough to cause significant errors. The situation is far more hazardous with high molecular weight polar molecules which may be irreversibly adsorbed. Since the physical behaviour of these molecules is usually unpredictable it is safest to burn such samples, analysing the products of combustion in one of several proven scintillation cocktails. The partial digestion of such macromolecules yields a spectrum of unknown fragments whose physical properties cannot be predicted. The virtue of sample combustion lies not so much in the opportunity to count these homogeneously as in the fact that the radionuclides are in a known chemical form for which a suitable counting system is available.

Two major advances in combustion instrumentation, the flow oxygen flask of

Kaartinen and the high speed combustion train of Petersen, go a long way towards overcoming objections to this method of homogeneous sample preparation.

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DISCUSSION

L.A. Wegner: You have presented a few results concerning the memory effects of commercially available combustion systems. What are the corresponding figures for the 'Pregl-chain' combustion system installed in your lab? What is the procedure involved to determine these values in respect to your choice?

B.E. Gordon: We simply run a blank after a sample and count the blank. For tritium we pass through about 0.25 ml water, for Carbon-14 we burn about 0.25 g hexadecane. Residual memory effect is in the region of a few-tenths of a percent. When combusting Carbon-14 samples the memory effect is of the order of 1/100 of a percent.

N.G.L. Harding: In viewing matters from this side of the crematorium it is apparent that different tissues combust at different rates (per unit mass). Tissue factors apparently determining the burning rate include water and salt content, tissue structure and fat content. Hence different samples require different combustion temperatures and oxygen concentrations. Do you know whether anyone has explored the possibility of optimising combustion temperatures using low-thermal-inertia systems such as a laser?

B.E. Gordon: Little work seems to have been done on the optimisation of combustion temperatures. Control of the combustion atmosphere is important.

B.W. Fox: I must stress that there are basic differences between true solubilisers and those mixtures containing surfactants and they should not be confused. A number of these mixtures described this morning contain surfactants and they belong to this afternoon's session.

M. Cosandey: Have you any information about cold combustion with Fenton's reagent, i.e. concentrated hydrogen peroxide with a trace of Fe^{2+} ion? In a recent report by IAEA, Sansom reported that all organic materials such as cationic exchange resins could be mineralised at low temperatures.

B.E. Gordon: I have not heard about this report or about this technique.

J.D. Eakins: With reference to the problem of controlling the rate of combustion in the furnace tube method of oxidation, we have overcome this at AERE, Harwell by the use of a nitrogen blanket. We use a single furnace tube on which two furnaces are wound. The first of these is for heating the sample and the second contains a platinum/aluminium catalyst maintained at 700°C . Oxygen is fed in between the two furnaces. Up to 10 g of organic sample, contained in a silica boat, is inserted into the cold sample furnace. Nitrogen is passed over the sample and the temperature gradually raised to 500°C . The volatile components of the sample are mixed with oxygen and flushed over the preheated catalyst where they are decomposed. The nitrogen blanket prevents ignition of the sample and a subsequent uncontrolled rise in temperature. When all the volatile components are evolved, oxygen is passed over the carbonised residue to complete the oxidation.