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A SENSITIVE, LOW BACKGROUND DETECTOR FOR RADIO GAS-LIQUID CHROMATOGRAPHY*

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SUMMARY

The design of a novel detector assembly for radio gas-liquid chromatography is described. The assembly comprises a gas flow proportional counter, plastic phosphor anti-coincidence guard counter and graded gamma shield. The net background count rate is 1.01 c.p.m.

The operational advantages of low background detector systems are discussed and the merits of digital display of counting information described.

INTRODUCTION

Radio gas-liquid chromatography (radio-GLC), a technique which seems likely to prove one of the most powerful available to the biochemist concerned with problems of metabolism, has been the subject of recent reviews by JAMES[1], KARMEN[2], and SCOTT[3].

The methods used to monitor the effluent stream from a GLC column for radioactivity fall into two main groups, those depending on the intermittent trapping of the effluent and those using continuous flow monitors. The former method, in which, characteristically, an automatic fraction collector is used to trap the effluent in cartridges filled with silicone-coated anthracene[4] or *p*-terphenyl crystals[5] which are then counted in a liquid scintillation spectrometer, has the advantage that samples may be counted over long periods of time. It suffers from the fact that the resolution offered by the fraction collector is intrinsically much lower than that of the GLC column; further disadvantages of this method lie in the risk of loss of material during fraction changing and in the high cost of the necessary ancillary liquid scintillation spectrometer. Continuous flow counters, in contrast, have the commanding merit that radio assay is made simultaneously with mass determination; comparison of the profiles of the mass and radio peaks, which is thus facilitated, provides a valuable indication of radiochemical purity and identity. A further advantage of most con-

* An apparatus of this design is now being manufactured by Panax Equipment Ltd.

tinuous monitors lies in the slightness of the losses encountered on dealing with carrier-free radio-labelled compounds. The major limitation inherent in the method is that the time during which effluent materials are available to the counter is restricted by the need to select such flow-rate counter–volume parameters as will prevent mixing of adjacent peaks.

Flow counting procedures in which the effluent peaks from the GLC column are absorbed continuously in liquid scintillation phosphor[6,7] or silicone oil-coated anthracene crystals[8] and the accumulated materials monitored by single or twin photomultiplier tubes operating in coincidence have been described; they share the serious disadvantage that statistical considerations make it impossible to detect minor peaks following major radioactive components. True flow-counting methods have been based on the use of ionisation chambers[9–11], scintillation spectrometers[5,12] and gas flow proportional counters[13] to detect the passage of radioactive peaks. The latter procedure has been extensively developed by JAMES *et al.*[1,14–16], who have advocated combustion and reduction of the effluent radio-peaks to $^{14}CO_2$ and $^3H_2$; the argon carrier is mixed with metered quantities of 'cold' CO_2, and the counting gas thus formed is fed to a gas flow proportional counter. By this means, the disadvantages of risk of contamination and of inherent instability of proportional counters operating at high temperatures are avoided. A variant of this procedure, in which the effluent is reduced to $^{14}CH_4$ and argon–methane used as the counting gas, has been described[17].

The resolution of a flow counter is determined by its volume and by the carrier gas flow rate; for any given set of resolution parameters, the sensitivity is determined by the counting efficiency and by the background current or count rate. The ionisation detectors used for radio-GLC offer counting efficiencies for ^{14}C and tritium of 33% and 75% respectively and show background currents of approximately 3×10^{-16} A, equivalent to the signal produced from a sample of activity 780 d.p.m.[11]. Large volume ionisation chambers offer higher counting efficiencies, but because of their lower resolution have found application only in interrupted elution radio-GLC[18]. The flow scintillation counter gives efficiencies of 70% and 20% for $^{14}CO_2$ and $^3H_2$ with background count rates in the respective channels of 15 and 50 c.p.m.[12]. Proportional counters used for radio-GLC are known to have counting efficiencies approaching 100% for $^{14}CO_2$ and in excess of 60% for $^3H_2$; background count rates are, of course, dependent on the type of construction and the extent of shielding but are commonly in the region of 25–50 c.p.m.[13,16,19,20]. The continuous flow, cylindrical window proportional counter which has been described[21] has a low counting efficiency for soft β emitters but has the advantage of ease of decontamination.

The present communication describes the design and performance of an anti-coincidence, gas flow proportional counter, having a background count rate of approximately 1 c.p.m. and able, in consequence, to detect low levels of radioactive materials in the effluent of GLC systems. It is currently in use in these laboratories for the identification and analysis of trace quantities of ^{14}C- and ^3H-labelled steroid metabolites in fish tissues.

APPARATUS

The detection assembly consists of a gas flow proportional counter, a cosmic guard counter, a graded shield, an anti-coincidence gate circuit and the data display

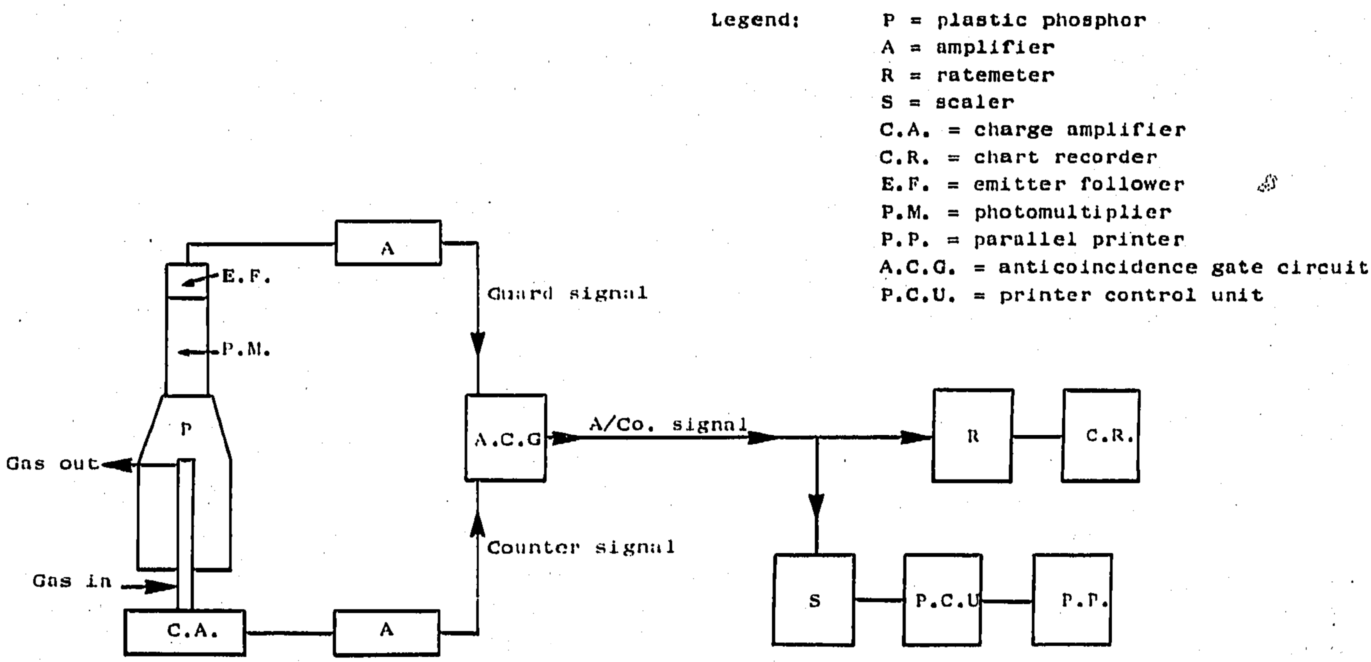

Fig. 1. Block diagram of counter and data presentation modules.

modules. These are described in detail below, and illustrated in block diagram form in Fig. 1. The signal from the proportional counter is fed via a charge amplifier and pulse amplifier to the input of the anti-coincidence gate. The cosmic guard counter comprises a cylinder of plastic phosphor, optically coupled to a photomultiplier whose output signal is fed, via an emitter follower and pulse amplifier, to the gate input of the anti-coincidence circuit. The latter rejects those signals from the proportional counter which are time-coincident with those from the guard channel. The anti-coincidence signal is then fed to a ratemeter and chart recorder. When accurate quantitation of the radio peaks is desired, this form of data display is supplemented by printed digital presentation of counts from a scaler/timer which may be interrogated manually, or automatically at pre-determined time intervals. The signal and guard counter are surrounded by a gamma ray shield of graded construction.

Details of the construction of the gas-flow counter are given in the dimensioned diagram, Fig. 2. The cathode of the counter is formed by a length of oxygen-free, high-conductivity copper (B.S.S. 1861). A tapered copper ring, fixed with epoxy resin at the top of the counter, provides a seat for the end shield; the lower flange forms the upper half of the gas seal. The counter is electroplated with high-purity nickel to prevent "tailing" of tritium peaks and the inside is polished by lapping. The anode wire (tungsten; 0.002 in. diam.) is located by two nickel-plated copper supports which serve, additionally, to reduce the electric field in the region of the teflon insulators. It is secured, at the upper end, by a teflon plug pressing in to a tapered hole in the anode support; a teflon cap is interposed between this plug and the end shield and covers the end of the wire. The wire is secured at its lower end by the central pin of a standard Gremar connector (Nuclear Chicago). The plated counter tube is a press-fit into a nickel-plated copper barrel which is threaded at its lower end to accept the Gremar connector coupling the counter to the charge amplifier. This arrangement

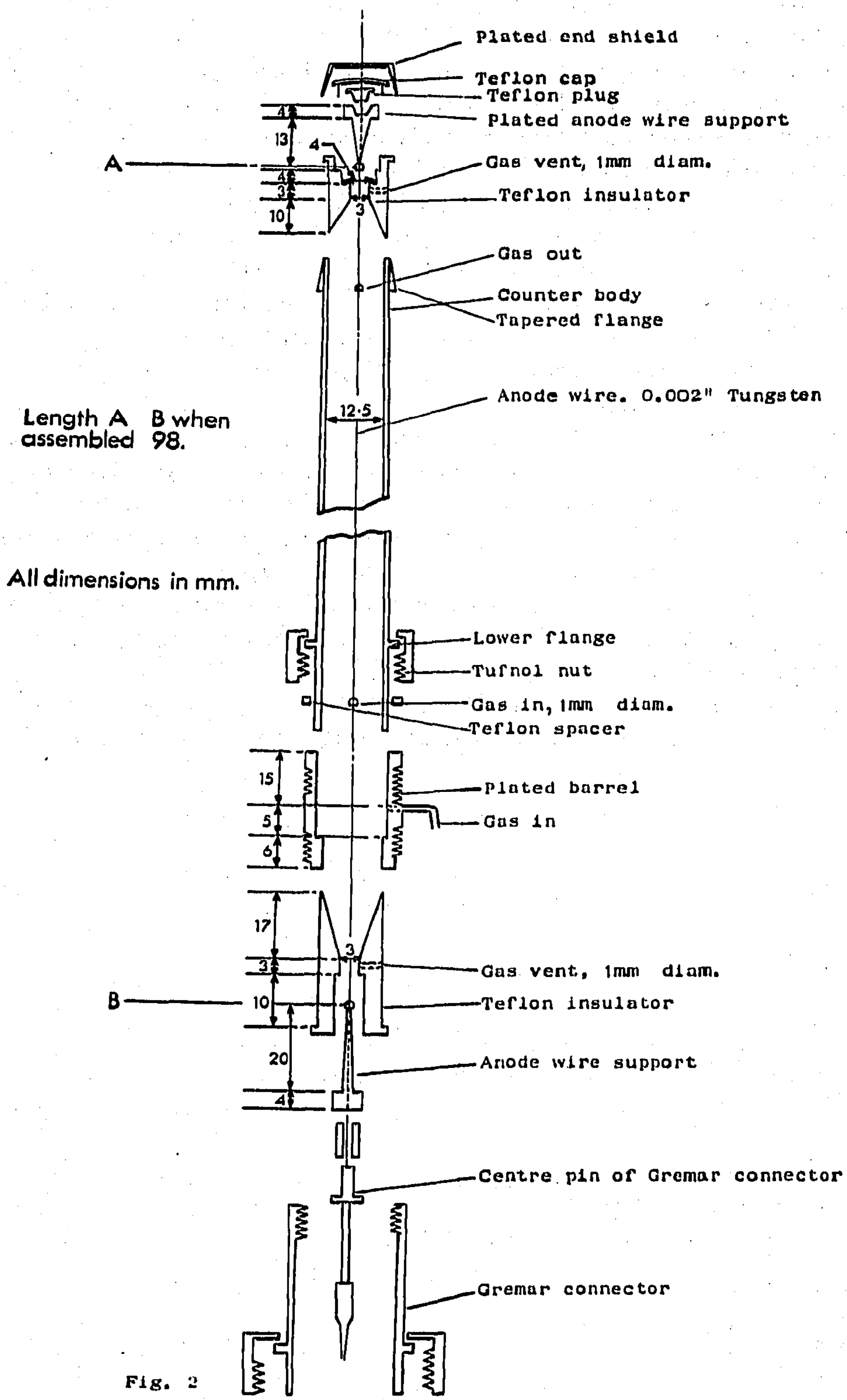

Fig. 2. Gas flow proportional counter.

ensures minimum degradation of pulse shape due to stray capacitances. The barrel conducts counting gas to the detector via a tapered teflon insulator; a Tufnol nut compresses a teflon spacer between the upper face of the barrel and the lower face of the counter flange to provide a gas-tight seal. The volume of the cell, 12 ml, was chosen as offering a satisfactory compromise between the competing requirements of achieving high count rates and of maintaining reasonable resolution.

The guard counter consists of a rod (8 in. × 3 in. diam.) of plastic phosphor (Naton 102A; Nuclear Enterprises) tapered over the upper 3 in. of its length to form an upper face, $1\frac{3}{4}$ in. diam., to which a photomultiplier (E.M.I.; type 6097S) is optically coupled by silicone oil. An axial hole ($\frac{5}{8}$ in. diam. × 5 in.) forms an inverted well for the gas flow counter from which exit gases are ducted via a radial hole, $^1/_{16}$ in. diam. The circumference and base of the phosphor are coated with titanium oxide paint to improve light collection by the photomultiplier.

The phosphor and photomultiplier are contained in separate, mating housings of tubular steel (pre-1945; wall thickness, $\frac{7}{8}$ in.), the lower of which is lined with high conductivity copper, $\frac{1}{4}$ in. thick. The proportional counter is inserted through this housing and into the well of the phosphor via a light-tight seal. Lead annuli (wall thickness, 2 in.) surround the two component housings and are supported by a lead-lined steel cave. An axial hole ($\frac{1}{4}$ in. diam.) drilled in one of the annuli allows an external ^{137}Cs source to be inserted, permitting the ready determination of counter plateaux. The shielding arrangements are illustrated in Fig. 3.

The emitter follower, charge amplifier, pulse amplifiers and ratemeter are standard, commercially available modules (Panax Equipment). The anti-coincidence

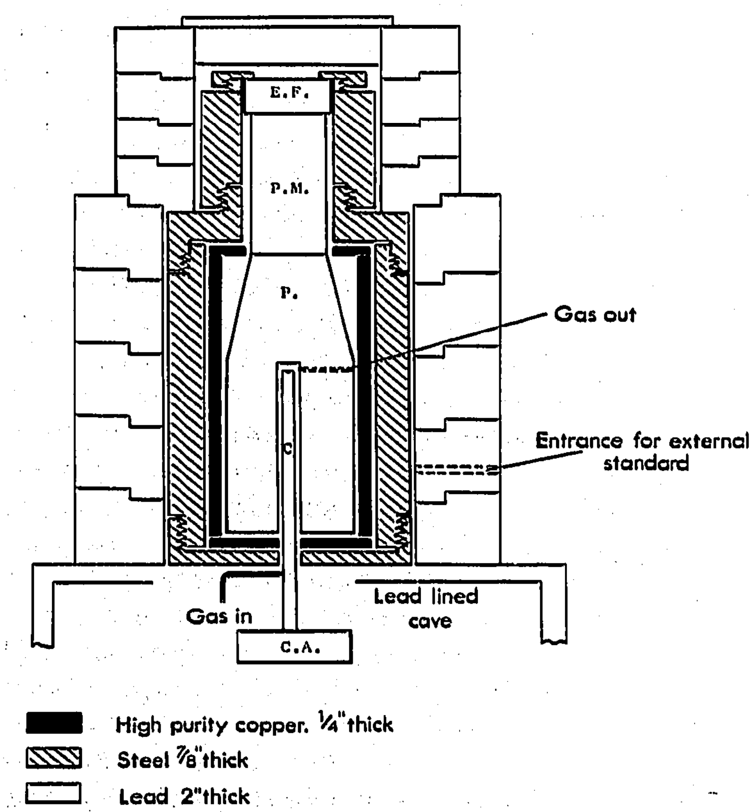

Fig. 3. Flow counter, cosmic and gamma guards.

circuit (Panax Equipment) is adjusted to provide optimum performance with the present counters; the signal pulse delay is 1 μsec and the width 1.5 μsec. The guard pulse width is stretched to 6 μsec.

Where digital presentation of counts is desired, to supplement the rate-meter–chart recorder display, a high-speed printer (Kienzle type KN66) is used[22] in conjunction with scaler, timer and printer control modules (Panax Equipment). This arrangement of digital display resembles that described by STÖCKLIN *et al.*[23].

PERFORMANCE

Counter characteristics

An investigation of the dependence of count rate on applied H.T. voltage was made using argon–CO_2 (95:5) as the counting gas and the external ^{137}Cs source. A count rate plateau was observed extending effectively (15% variation) from 1450 V to 1825 V; in the central region of this (1600–1750 V) the variation of count rate with H.T. did not exceed 1%/100 V. An exactly similar plateau was obtained when an internal source of poly(methyl-^{14}C) methacrylate was used in place of the external gamma standard.

The counter efficiency was determined by releasing into the argon–CO_2 gas stream known quantities of $^{14}CO_2$, prepared by injecting standardised aqueous $Na_2{}^{14}CO_3$ into dilute sulphuric acid contained in "bubbler" set in the gas stream. The counting efficiency (E) was calculated from the expression:

$$E\,[\%] = \frac{\text{observed counts} \times 100\,F}{\text{applied activity [d.p.m.]} \times V}$$

where

F = flow rate (ml/min)
V = counter volume

The results of replicate determinations indicated that the efficiency for ^{14}C was 94.5% (S.D. $\pm$ 1%). The apparent efficiency, which was derived when the active volume (volume of the cylinder whose axis is the anode wire) was used in place of the total cell colume in the above expression, was 101.5%; this value reflects the fact that electrons from gas particles in the cone sections are detected, though at less than 2π geometry.

Efficiencies of ^3H were determined by injecting standardised solutions of *n*-hexadecane-1,2T(*n*) on to the gas chromatograph; the leg from the effluent splitter to the flame detector was blanked off during these determinations. The mean, observed efficiency was 64% (S.D. $\pm$ 1.5%).

Background count rate

The background count rate of the unshielded proportional counter was found to be 38.7 c.p.m. (S.D. $\pm$ 0.3 c.p.m.); this was reduced to 15.4 c.p.m. (S.D. $\pm$ 0.3 c.p.m.) by the lead–steel–copper shield. Determinations of count rate during a period of 48 h indicated that this background was reduced to 1.01 c.p.m. (S.D. = $\pm$ 0.02 c.p.m.) by the anti-coincidence guard; the coincidence component, presumably originating largely in cosmic events, was approximately 8.6 c.p.m. This proportion of cosmic to total counts is in substantial agreement with values quoted by other workers[24–26]. Table I summarises the effects of shielding and cosmic guarding on the background count rate. The results of Pearson's Chi square[27] test, shown in column 4 (P), indicate that the background counts fit a Poissonian distribution. Changes in the H.T. supply voltage to the photomultiplier which caused changes in the count rate of the guard detector from 200 c.p.m. at 800 V to 1680 c.p.m. at 1150 V did not significantly alter the anti-coincidence background count rate.

TABLE I

EFFECTS OF SHIELDING AND COSMIC GUARDING ON THE BACKGROUND COUNT RATE

Shield	*Background (c.p.m.)*	*S.D. (c.p.m.)*	*P*
—	38.7	± 0.3	
Steel–copper	15.4	± 0.3	
Ditto + anti-coincidence guard	7.05	± 0.3	
Lead–steel–copper	9.61	± 0.2	
Ditto + anti-coincidence guard	1.01	± 0.02	0.2

In an early form of the counter which had no plated copper end cap, the anti-coincidence count rate was low ($\simeq$ 0.3 c.p.m.), but at the expense of a sharply lowered counting efficiency. The fact that the background rate was dependent on both counter and photomultiplier H.T. (decreasing with increasing voltage) made clear the necessity of an end cap to prevent photons in the proportional counter from being "seen" by the photomultiplier and causing a loss in desired counts.

Results of experiments with a variety of counters of different arrangements of anode wire location, showed the importance of the plated copper supports in reducing spurious events, presumably by reducing the electrical field in the region of the insulators. In the absence of these supports, the anti-coincidence background count rate was raised to approximately 4 c.p.m.

Operation

The counter is currently in use with an F & M 400 Biomedical gas chromatograph, fitted with an effluent splitter. The output from the rear port is combusted and reduced in a furnace tube containing copper oxide and iron, as described by JAMES *et al.*[1,14–16]. Carbon dioxide (5%) is added at the auxiliary port to form a counting gas from the argon carrier and hydrogen is injected into the furnace train to maintain the iron in a reduced form[5].

DISCUSSION

The confidence with which the events counted by a radio-detector may be expected to lie within stated deviations from the expected value is given by the familiar expression:

$$d^2 = \frac{10^4 K^2}{T} \left(\frac{1}{NE} + \frac{2B}{N^2 E^2} \right)$$

where

d = % deviation
K = confidence constant
T = counting time
B = background count rate
N = sample disintegration rate
E = counting efficiency

When the radioactive effluent of a GLC is monitored discontinuously, by trapping, the background count rate and efficiency of the counter do not, in principle, set limits to the sensitivity of the radio-detection system since appropriately long counting times may be chosen. Under flow detection conditions, however, T is the mean residence time of gas particles in the counter, expressed by V/F where V is the counter volume and F the flow rate. Given that these parameters are fixed by the need to obtain some desired resolution and that the efficiency of a counter is normally an inherent property, the sensitivity of detection of radioactive materials from a GLC column by flow counting may be improved only by reducing the background count rate. Flow counting techniques have such commanding advantages, save in sensitivity, over discontinuous methods that reduction of the background is of considerable importance.

Of the three types of radio-detectors—ionisation chamber, scintillation spectrometer and proportional detector—which have been used as continuous-flow counters, the last seemed most likely to respond to efforts to reduce the background count rate. This background is compounded of contributions from spurious events associated with the counter insulation, from the natural radioactivity of the counter construction materials, from cosmic events and from ambient gamma radiation. The counter described in the present communication was constructed of materials of the lowest intrinsic radioactivity and spurious events were minimised by reducing the electrical field in the region of the insulators. Its gamma shield was of compound form, constructed of materials of decreasing Z number, in order to reduce secondary gamma radiation originating in the shielding as a result of cosmic events; the reduction in the background of the proportional counter caused by this shield was comparable with that effected by single shields of much heavier construction[28]. Plastic scintillator materials have found recent application as anti-coincidence shields[28–30]; the choice of this type of cosmic guard counter in the present detector assembly was dictated by its considerable advantage in ease of maintenance and convenience of geometry over the conventional Geiger-Müller umbrella and by its useful, though small, guarding efficiency for residual gamma radiation. The short width of the guard pulse generated in the anti-coincidence circuit, ensured that, over the range of guard count rates encountered, the loss of desired counts from the proportional detector is negligible ($< 0.05\%$).

The sensitivity of detection of radio peaks from a gas chromatograph depends, of course, on the retention volume of the material being examined. In the following comparison of the sensitivities of different detectors, it is assumed that the active material enters the counter as a bolus which retains a flat-topped, rectangular activity profile as it passes through the counter—conditions which are only approximately realised under actual conditions; it is further supposed that the gas flow rate is adjusted to provide a mean residence time in the counter of 1 min. The counter described in the present communication has a mean background of approximately 1 c.p.m. and the probabilities that during any single measuring interval of 1 min, 0, 1, 2, 3 ....r counts are recorded are given by e^{-1}, $e^{-1}/1!$, $e^{-1}/2!$, $e^{-1}/3!$ ....$e^{-1}/r!$. There is thus a probability, given by $\sum_{r=4}^{\infty} e^{-1}/r!$ and approximately equal to 0.02, that a count of 4 or more, during any single minute, is due merely to background. Assuming that the efficiency of the counter is 100%, the probability that a bolus of radioactive material, of mean disintegration rate m d.p.m., will cause a count of 4 or more in any single minute is given by $\sum_{r=4}^{\infty} (m + 1)^r e^{-(m+1)}/r!$. From this, it may be calculated that the smallest radioactive sample for which there is a 95% probability that it will be detected (by causing 4 or more counts per minute) is 6.7 d.p.m. Using the same probabilities as before, a counter whose mean background count-rate is 50 c.p.m. would detect a radioactive sample of 30 d.p.m. Lowering the background of the counter from 50 to 1 c.p.m. has effected therefore an approximately five-fold improvement in its sensitivity

Data from a radio-GLC system are commonly displayed by a ratemeter and chart recorder; this form of presentation, though in many ways attractively direct, has some inherent disadvantages. The factors which determine the ability of a ratemeter to present faithfully the profiles of radioactivity in the effluent of a chromatograph are the normal statistical variation of radioactive emission, the volume of the counter, the rate of gas flow and the electrical and mechanical inertia of the ratemeter and recorder. These are strictly inter-related. If too short a ratemeter time constant is chosen, counting information will be obscured by the rapid changes in the displayed count rate caused by the random nature of radioactive emission; if too long a time constant is chosen, counting information will be irretrievably lost by the failure of the ratemeter to respond with sufficient speed to changes in the frequency of events within the counter. These factors may be considered in detail as follows. A ratemeter is essentially an averaging instrument which takes a finite time, t, to respond to changes in the rate of input pulses. This time is determined by the expression

$$t = RC\,[\tfrac{1}{2}\log_e 2n'RC + 0.394]$$

where

RC = ratemeter time constant
n' = change in input count rate

In practice, it has been found that this may be approximated to

$$t \geqslant 4\,RC$$

Clearly this equilibrium time t must be less than the mean residence time, t_1, of gas in the counter if the ratemeter is to reproduce faithfully the profile of counts registered by the detector. RC in turn determines the probability that the ratemeter reading lies within certain defined deviations from the mean count rate, according to the expression

$$RC = \frac{K^2\,10^4}{2N\,d^2}$$

from which it follows that

$$d^2 \geqslant \frac{2K^2\,10^4}{Nt_1}$$

In contrast, consideration of the statistics of data presentation by a scaler leads to the expression

$$d^2 = \frac{K^2\, 10^4}{N t_1}$$

Thus the size of sample necessary to obtain a determination of radioactivity to within the same limits of deviation and with the same counter-volume and gas flow rates is, with scaler presentation, just half that necessary when the data are displayed by a ratemeter.

Although integrating ratemeters do not suffer the statistical disadvantages of the normal ratemeter and have been used by some workers to display radio-GLC data, their operation is considerably less convenient than the digital system described in the present communication.

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