

AUTOMATIC RECORDING OF THE RADIOACTIVITY OF ZONES ELUTED FROM THE GAS-LIQUID CHROMATOGRAM

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The high resolving power of the gas-liquid chromatogram has prompted many attempts at designing counters capable of continuously recording the radioactivity of vapours eluted from the column. Flow proportional counters working at column temperature have been used¹⁻³, as has a proportional counter at room temperature⁴. In the latter case condensation of the zones was prevented by dilution with a large volume of gas introduced into the train after the vapour detector. Scintillation counting has been adopted by KARMEN AND TRITCH⁵ by condensing fractions in tubes containing a crystalline scintillator coated with a silicone oil. POPJAK and his collaborators⁶ designed an ingenious trap containing a solution of a scintillator in toluene held between two photomultiplier tubes. All the zones accumulate in the trap and an automatic range-changing device keeps the record of count rate on the scale of the recorder. In our experience these methods possess some disadvantages. Apart from the general difficulties of construction of proportional counters capable of operating at temperatures up to 250°, expansion of the centre wire can change the operating characteristics. A major problem is the poisoning of the centre wire caused by stripping of stationary phase from the column. Dilution of the gas stream from the detector with cold gas when long-chain fatty acids are being separated can lead to deposition within the cell.

Trapping of individual fractions followed by counting either with an end-window counter⁷ or with a scintillation counter⁵ is laborious and necessary only when substances of very low specific activity are to be detected. Furthermore, fractions can be conveniently collected only when the vapour detector records a peak; radioactive intermediates present in very small amounts can be missed. The apparatus of POPJAK⁶ is efficient but suffers from the disadvantage that all the zones are trapped in the cell so that fast moving materials of high specific activity can cause the instrument to switch to low sensitivity unless the cell is emptied and refilled during the separation.

We have adopted the alternative approach of using argon as the carrier gas of the chromatogram and burning all organic compounds (whether derived from the stationary phase or an eluted zone) over heated copper oxide. Water is removed by passing the gas stream over coarsely powdered magnesium perchlorate; carbon dioxide is injected to give a final concentration of 5% and the gas is passed, at room temperature,

into a proportional counter of the simplest construction. To detect and record the pulses from the proportional counter a linear amplifier, ratemeter and pen recorder are needed. The amplifier (for ^{14}C) has a gain of 200 and can conveniently be a "ring of three" in a small case which also contains an E.H.T. filter, adjacent to the counter. This unit derives its electrical power from the ratemeter. The ratemeter, which includes an E.H.T. supply, has a pulse-height discriminator, followed by the usual pulse shaping circuits, diode pump integration and valve voltmeter stages. For the presentation of pulses occurring at rates less than a few pulses per second the "rate" presentation is unsuitable because the relatively long integration times, which are necessary to maintain a slowly changing value for recording, limit the resolution. A more satisfactory record is obtained by integrating the peaks (Fig. 6B). The ratemeter used in this apparatus was modified to give four ranges of rate (1, 10, 100 and 1000 pulses/sec) and three integrating ranges (50, 500 and 5000 pulses) with provision for any intermediate setting. In addition, a circuit was provided allowing automatic shorting of the integrating capacitor when full-scale deflection was reached.

The apparatus is cheap and easy to construct, reliable in operation, the counter has almost 4π geometry and can be used with conventional commercial counting equipment.

EXPERIMENTAL

Construction of gas train

A silica tube $\frac{1}{2}$ in. diameter and 2 in. long is attached to the vent of the vapour detector by a P.T.F.E. connector. Around the silica tube is placed a small furnace (Fig. 1) of the type used to vaporise the sample in a Dumas combustion train. A

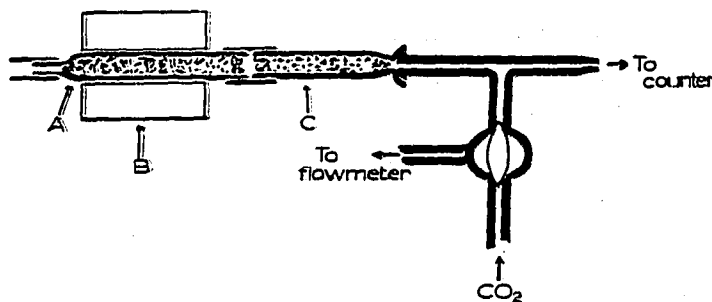


Fig. 1. General arrangement of the gas train for combustion and drying of the vapour eluted from the gas chromatogram. A: Silica tube packed with copper oxide (wire form). B: Furnace. C: Drying tube containing anhydrous magnesium perchlorate.

furnace temperature of approximately 700° is suitable. The cool end of the silica tube is attached with silicone rubber tubing to a $\frac{1}{4}$ in. diameter glass tube containing coarsely powdered anhydrous magnesium perchlorate. The end of this tube is fitted with a spherical ground glass joint allowing a flexible attachment to a capillary T-piece in which the CO_2 is introduced. The gas stream then passes through a short length of silicone rubber tubing to the entry port of the counter. A trap containing soda-lime is attached at the exit tube of the counter to absorb all the CO_2 .

Construction of the counter

The body of the counter consists of a short length (most frequently 4 in.) of $\frac{1}{2}$ in. copper water pipe. The pipe possesses a very smooth interior finish and requires no treatment beyond acid dipping. The end plugs are machined from polythene, the centre of each is tapped 2 B.A. thread to take the brass anode supports, which also serve to introduce the gas (Fig. 2). Contact between the anode wire (0.002 in. diameter tungsten) and the support, is achieved by bending the wire over the tube and



Fig. 2. Section of proportional counter. A: Tungsten wire 0.002 in. diameter. B: Polythene plug. C: Brass insert. D: Copper tube $\frac{1}{2}$ in. diameter.

forcing on a short length of silicone rubber tubing. The anode connection to the amplifier is soldered to one of the anode supports. The counter is assembled as follows: one of the polythene caps is inserted, the tungsten wire is drawn through and held in place by the rubber tubing. The wire is drawn through the counter tube and then through the other cap, the cap is forced into place, the wire is pulled tight, bent over the brass insert and silicone rubber tubing pushed over the insert. The counter is contained in a 1 in. thick lead sandwich to which is attached the amplifier.

Circuitry

Amplifier. Many commercial amplifiers are suitable, two known to the authors are as follows:

Burndept BN 106 and Dynatron N 640, with the gain adjusted to be $\times 200$.

Ratemeter. The ratemeter used was an A.E.R.E. type 1037b, the commercial equivalent is the Dynatron N 522. The higher ranges of rate originally provided on the instrument were not required for this work and these positions were used to

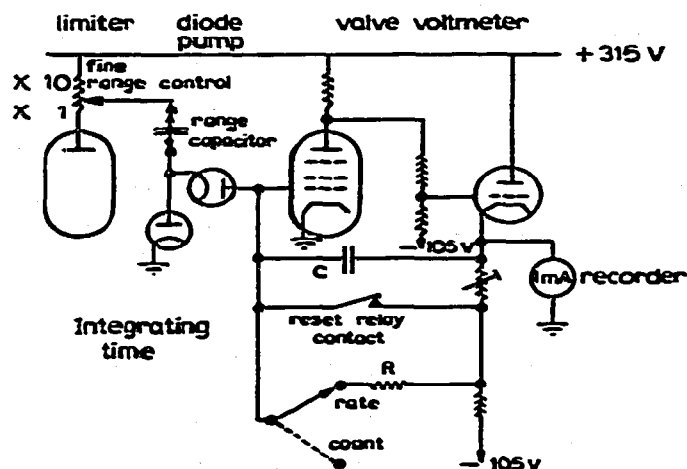


Fig. 3. Circuit illustrating range switching in the ratemeter.

provide three integrating ranges of 50, 500 and 5,000 counts. A fine control provided a continuous change of range sensitivity between the coarse steps (Fig. 3). The integrating capacitor is automatically shorted by a relay contact when full scale deflection is reached, the relay being operated for a time sufficient to discharge the condenser via a flip-flop (Fig. 4) to ensure that the capacitor is fully discharged. The relay can be operated by switch contact in the case of a servo recorder or with many coil recorders the pointer can intercept a light beam falling on a photo-transistor.

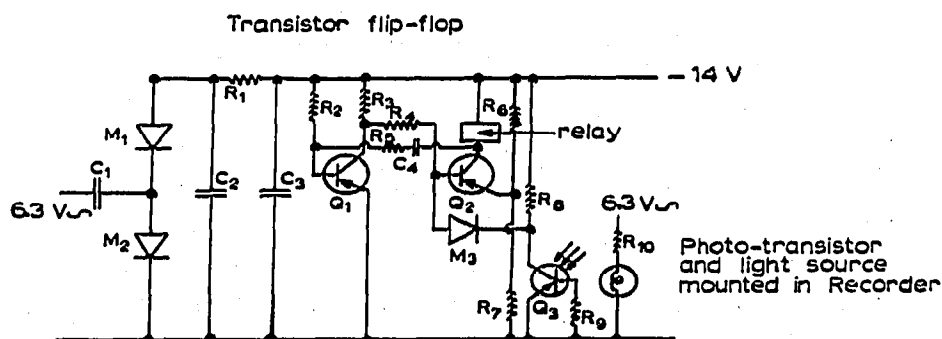


Fig. 4. Circuit for automatic shorting of integrating capacitor actuated by full scale deflection of the recorder.

M _{1, 2 and 3}	Mullard OA 71	R ₁	470 Ω	} all 1/4 W 10%	R ₆	12 k Ω	} all 1/4 W 10%
Q _{1, Q2}	Mullard OC 71	R ₂	560 k Ω		R ₇	1.5 k Ω	
Q ₃	Mullard OCP 71	R ₃	10 k Ω		R ₈	47 k Ω	
C _{1, 2 and 3}	25 μ F 50 V electrolytic	R ₄	47 k Ω		R ₉	10 k Ω	
C ₄	2 μ F 25 V electrolytic	R ₅	56 k Ω		R ₁₀	33 Ω 3 W vitreous	

Relay 14,000 Ω 0.8 mA operating current.

Calibration

(a) *Counter plateau.* An external radiation source can be used to determine the plateau of the counter. The ratemeter and recorder are set to record count rate while the E.H.T. voltage is increased in small steps until the discharge point is reached. Inspection of the record will show the position and length of the plateau; the operating point corresponds to the voltage at the centre of the plateau. This procedure should be repeated occasionally to check on counter performance.

(b) *Counter efficiency.* Stearic acid of known specific activity was diluted with inactive stearate and the absolute activity was determined by combustion according to the procedure described by BRADLEY *et al.*⁸. Samples of the methyl ester of the acid were then run on the gas chromatogram and the counts obtained from known quantities compared with the known absolute count.

RESULTS AND DISCUSSION

Typical recorder diagrams are shown in Figs. 5 and 6. The rate record (Fig. 5, curve B) is useful when scanning a group of acids for the occurrence of radioactivity but less useful when an accurate measurement of amount of radioactivity is required. The

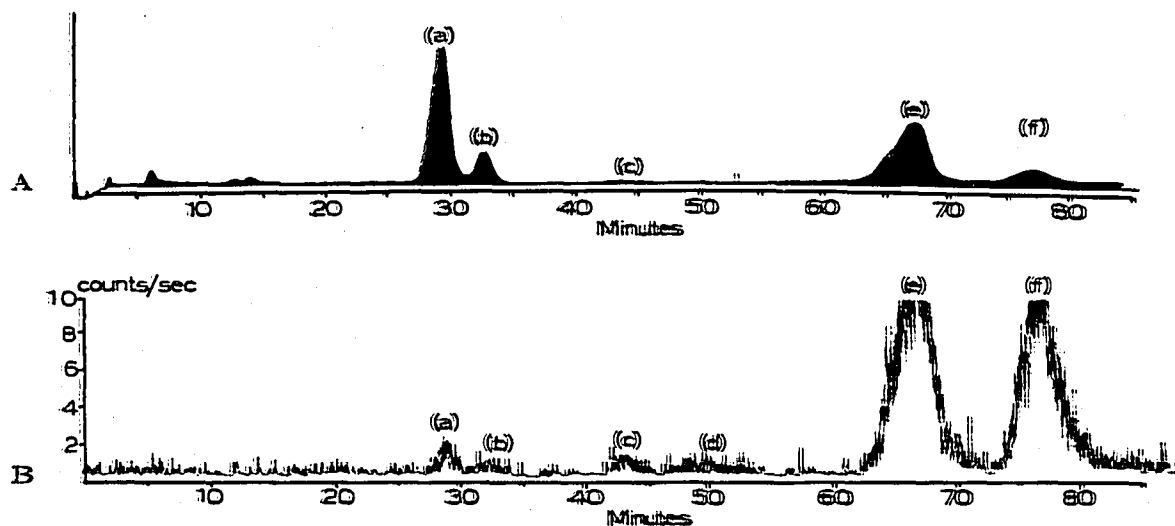


Fig. 5. Comparison of simultaneous record of vapour detector (gas density meter) and proportional count rate, from analysis of fatty acids isolated from yeast grown in the presence of $2\text{-}^{14}\text{C}$ -stearic acid, containing labelled palmitic and heptadecanoic contaminants. Peak identification: (a) Palmitoleic acid; (b) Palmitic acid; (c) *n*-Heptadecanoic acid; (d) *n*-Heptadecanoic acid; (e) Oleic acid; (f) Stearic acid. Labelled-palmitic and *n*-heptadecanoic acids were minor impurities in the labelled stearic acid. Column: 4 ft. Apiezon L on Celite 100-120 mesh at 197° . Argon flow rate 10 c.c. in 5.5 sec. CO_2 flow rate 0.5 c.c. in 5.5 sec. Load 1.0 mg.

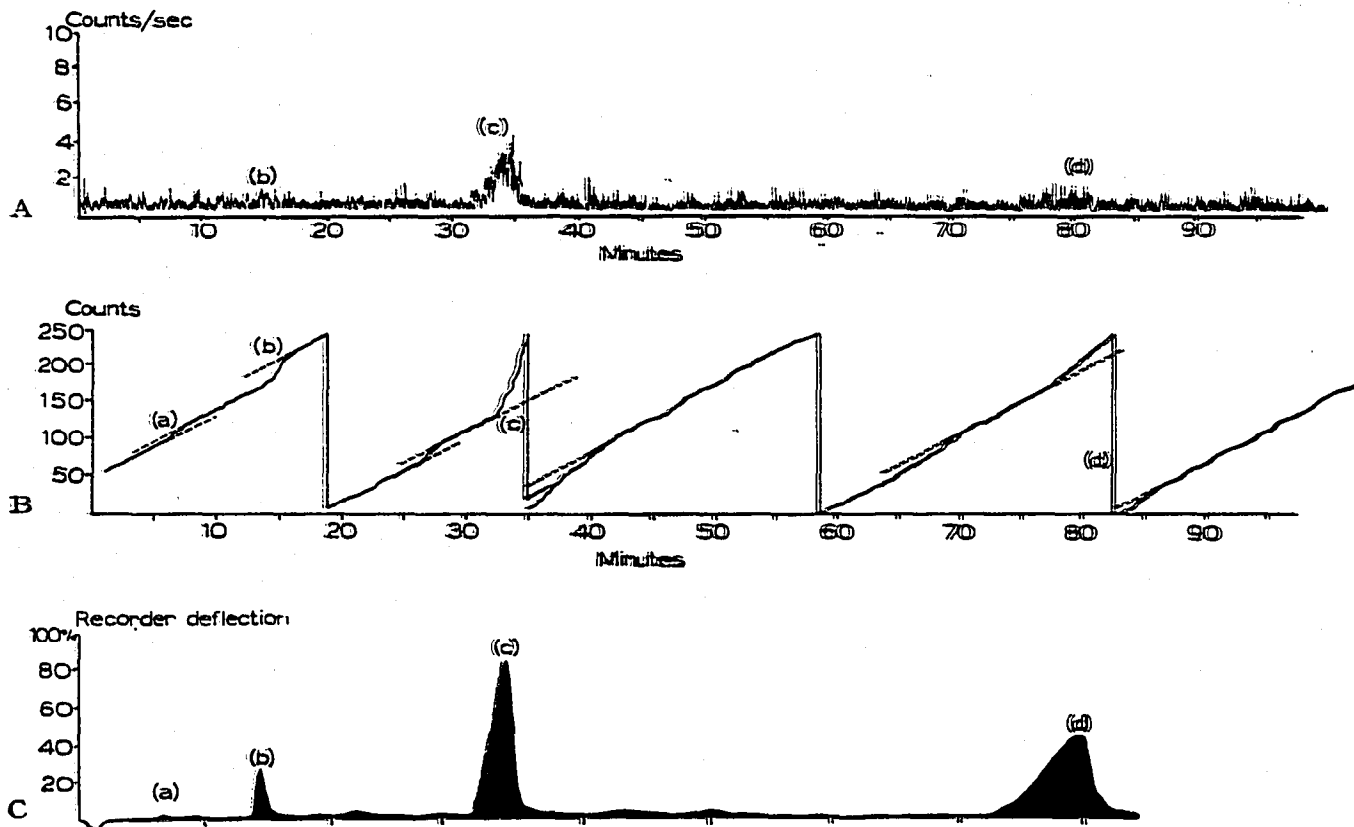


Fig. 6. Comparison of records of integrated count and count rate for a mixture of labelled lauric, myristic, palmitic and stearic acid. A: Count rate measurement; B: Total count measurement; C: Vapour concentration record. (a) Lauric acid; (b) Myristic acid; (c) Palmitic acid; (d) Stearic acid.

integrated count (Fig. 6, curve B) is best for accurate measurements since the count less background can be read off directly. The base line slopes upwards because of the accumulation of background count.

Sensitivity. The sensitivity of the counter varies inversely with the flow rate. With a volume of 10 c.c. the average residence time of each molecule at a flow rate of 2 c.c./sec will be 5 sec. This is adequate for specific activities of the order of 600 counts/min/mg and greater. The minimum detectable amount will vary with the retention volume of the peaks, slow peaks emerging over 10 min or more will require larger loads. The volume of the counter should not be greater than that of the vapour detector or peak resolution will suffer.

If used in conjunction with ionisation vapour detectors such as the hydrogen flame ionisation and argon ionisation instruments, a gas splitting device must be placed at the end of the column in order to allow the use of large (1 mg upward) column loads without overloading the detectors. In many biochemical experiments the amount of labelling is small so that a few mg of material will be necessary to obtain sufficient counts. It will often be an advantage to use column conditions (*i.e.* polar stationary phases and/or higher than usual column temperatures) to decrease the retention volume of the components so that low flow rates can be used and yet allow reasonable times of analysis.

The system should also be applicable to tritium counting. In this case the magnesium perchlorate should be replaced by a reagent such as calcium carbide to generate a permanent gas from the water produced by combustion of the sample. Greater amplification of the pulses would be necessary.

Counter efficiency. Comparison of the absolute count from a sample of labelled methyl stearate showed that 90 % of the counter volume was effective. By plotting counts for a known sample weight obtained at a variety of flow rates against the flow rate a straight line was obtained indicating complete mixing within the counter volume.

SUMMARY

A simple proportional flow counter for use with the gas chromatogram is described, which is operated at room temperature by burning all eluted material to CO₂ over heated copper oxide. The gas stream is dried, 5 % CO₂ is injected and the mixture passed into the counter. Details are given of the necessary circuitry. Examples are provided of its use with long chain fatty acids.

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