A Simple Helium-Ethanol Flow Counter for Monitoring Chromatograph-Column Effluents Containing Weak β -Emitting Isotopes

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The conventional method for assay of weak β -emitting isotopes in the effluent from a chromatographic column is to dry down portions of fractions on planchettes, which are then counted under a standard thin-window E.H.M.2.S. (G.E.C. Research Laboratories) tube counter. This technique is laborious and slow, although sensitive and quantitatively relatively accurate. Other ways of monitoring the liquid as it comes off the column, involving the use of the E.H.M. tube, have been described, but they are relatively insensitive because of the small area (5 cm.²) of the end-window. In the counter described below sensitivity is increased by running the liquid as a thin film in contact with a thin window seven times the area of the E.H.M. window.

CONSTRUCTION

The dimensions and assembly are apparent from Fig. 1. The window is of silicone-treated unsupported mica, which is replaceable, since the counter

1.5 cm. from the ends of the cell so that they are not immediately above unsupported mica; it is thought that this allows better liquid-flow characteristics. The polythene is very slightly recessed between the entry and exit tubes and the ends of the window, to allow liquid to flow through.

In assembly, after the counter has been cleaned carefully, a thin layer of silicone grease is smeared on the surface of the counter and the mica sheet laid on it over the cell. Another layer of grease is smeared over the edges of the mica, and the polythene gasket and backing plate are screwed on tightly. An effective seal (which is essential) is obtained by having milled flat steel surfaces, a rigid backing plate, a minimal amount of silicone grease and stout tightened screws. The liquid is thus in contact only with polythene and mica, and concentrated acids can be run through without corrosion. The liquid dead space is about 1.5 ml., including the entry and exit tubes; when a radioactive solution in the counter (giving 400 counts/min.) was washed through with 3 ml. of water a

Fig. 1. Scale diagram of helium-ethanol flow counter for use with ^{14}C or ^{35}S in a liquid.

is easily dismantled. Mica sheets 25 cm. $\times$ 3 cm., 2.5 mg./cm.², are available from the Essex Mica Co., Hainault, Essex. The effective area of the window is $2.5 \times 15 = 37.5$ cm.².

The body of the counter in use is of stainless steel, but it may be made of brass, and the dimensions are not critical. The polythene gasket is thick enough to mount two small connecting tubes for the entry and exit of liquid. These holes are offset

95% reduction in count rate was obtained, and after 10 ml. the count rate had fallen to background. N-HCl containing ^{14}C -labelled piperazine compounds (Bangham, 1955) has been run through the counter over the course of 2 weeks, and no radioactive contamination was noticed.

Helium bubbled through sodium sulphate-dried ethanol at 0° is passed through the counter cell at about 0.8 l./hr. This flow rate is not critical and

needs little attention. A lower rate is possible if a simple ethanol trap is connected to the exit pipe to prevent back-contamination with air.

The counter has a plateau of 1275–1775 v with a slope of 1.2 % per 100 v. With a working potential of 1400 v the counter is stable. The background is stable over long periods (about 50 counts/min. in a lead castle 1 in. thick).

The counter can be connected to a standard or log rate meter coupled to a pen recorder. A relay on the fraction-cutter siphon records each fraction on paper. The maximum rate of flow of the liquid depends on the radioactivity and the integrating time of the rate meter. With the geometry used and with ^{14}C in a liquid of specific gravity 1.0, approximately 1 % of disintegrations are recorded. This means that the counter gives the same number of counts as 0.2 ml. of the liquid evaporated and counted under an end-window Geiger-Müller tube. The counter must be calibrated each time it is reassembled, because the geometry of the liquid

film may be altered. The pressure of the liquid should be kept constant.

The counter provides a continuous, immediately available record of radioactivity coming off a column, and it obviates a large number of tedious sampling functions. It is not intended as an instrument for accurate measurements.

SUMMARY

A simple helium-ethanol flow counter suitable for indicating radioactivity due to weak β -emitting isotopes in the effluent from a chromatographic column is described.

I wish to thank Dr J. E. S. Bradley and Mr R. C. Holloway for their advice, Mr C. Doré for constructing the counter, and Mr K. R. Hobbs for his technical assistance. This counter was exhibited at the Second International Isotope Conference at Oxford, 1954.

REFERENCE

Bangham, D. R. (1955). *Brit. J. Pharmacol.* **10**, 397.

Studies in Carotenogenesis

20. CAROTENOIDS OF SOME SPECIES OF *CHLOROBIVM**

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No unequivocal information about the presence or absence of carotenoids in the green sulphur bacteria is available. van Niel & Muller (1931) failed to detect carotenoids in their cultures, but Katz & Wassink (1939) reported their presence in a different strain. It has now been possible to examine three species of *Chlorobium*; carotenoids were found in all and they have been separated and identified. A preliminary report of this work has already been given (Goodwin & Land, 1955). Barer (1955) has also recently mentioned the presence of γ -carotene in species of *Chlorobium*.

EXPERIMENTAL

Cultures. The three species of *Chlorobium* examined were *C. thiosulphatophilum* (kindly supplied by Dr June Lascelles, Dept. of Biochemistry, The University of Oxford), *C. limicola*, and an unidentified halophilic species, both kindly supplied by Mr K. R. Butlin, Chemical Research Laboratory, D.S.I.R., Teddington, Middlesex.

Culture medium. The medium used was that recommended by Larsen (1952). For culturing the halophilic

strain the NaCl concentration was increased from 1.0 to 2.5 % (w/v).

Diphenylamine media were made up from a quarter-saturated solution (1/140 000) of diphenylamine as the solvent instead of pure water.

Cultural conditions. The bacteria were grown anaerobically in completely filled glass-stoppered bottles at 30° in a glass incubator illuminated by two banks of four 60 w tungsten-filament lamps (Garton, Goodwin & Lijinsky, 1951). *Phycomyces blakesleeana*, required as a source of γ -carotene, was grown under conditions described by Garton *et al.* (1951).

Extraction and separation of pigments. The centrifuged cells were first extracted twice with methanol to remove chlorophyll (Polgár, van Niel & Zechmeister, 1944; Goodwin & Osman, 1953) and then twice with ethyl ether. The combined methanol fractions were saponified by the method of Goodwin & Morton (1946) to remove chlorophyll and lipids that might interfere with chromatography. The unsaponifiable matter was extracted, combined with the original ethereal extract, dried by standing over anhydrous Na_2SO_4 , evaporated to dryness *in vacuo* at 30° and redissolved in light petroleum (b.p. 40–60°) ready for chromatography (throughout this work the term light petroleum refers to a fraction with boiling point 40–60°).

The pigments were separated by chromatography on activated alumina (P. Spence and Co., Widnes, grade O),

* Part 19: Goodwin (1956).