A LIQUID SCINTILLATOR-BASED CONTINUOUS RADIO CHROMATOGRAM SCANNER

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INTRODUCTION

ROUCAYROL *et al.*[1] first adapted the principle of liquid scintillation measurement of radio-activity to the direct and continuous scanning of paper chromatograms carrying compounds labelled with isotopes which emit soft β-radiation. In spite of the considerable effort devoted in recent years to techniques for measuring soft β-activity on paper chromatograms this particular method appears to have received little further attention.

In general, the methods adopted for assaying radio-activity distributed in paper chromatograms fall into two classes. Continuous and direct scanning devices utilise either thin-window or windowless Geiger tubes. The efficiency of some of these devices is enhanced by the use of two coaxially mounted Geiger tubes and by a close approach to 4π geometry. A recently introduced instrument in this class was described by SHIPOTOFSKY[2], who also gives a survey of the relevant literature.

The second class of methods embraces all the techniques based upon the use of a scintillator which translates the energy of β-particles into light quanta. With the exception of the method of SELIGER AND AGRANOFF[3] and a recently introduced commercial instrument*, in which anthracene crystals serve as scintillators, the majority employ liquid scintillators. As conceived originally, the distribution of radio-activity was assayed by cutting the developed paper chromatograms into strips at right angles to the direction of solvent flow. These strips were then impregnated with the scintillator solution and individually placed upon the photomultiplier tube. A reflector of aluminium foil placed upon the strip enhanced the light output (SELIGER AND AGRANOFF[3], FUNT AND HETHERINGTON[4]). This technique was developed further by WANG AND JONES[5], who counted the activity of individual strips by immersing these entirely in liquid scintillator contained in a glass vial. The popularity of this technique grew with the introduction of automatic sample changing devices. It has been repeatedly discussed in the literature (LOFTFIELD AND EIGNER[6], SHERMAN[7] and BAXTER AND SENONER[8]).

With the notable exception of the instrument designed by ROUCAYROL *et al.*[1], none of the methods of liquid scintillation counting of paper chromatograms reported in literature permit continuous as well as direct scanning.

The instrument and method of scanning paper chromatograms developed in this laboratory takes the idea of ROUCAYROL *et al.*[1] a stage further. With the aid of

* Panax Ltd., Mitcham, Surrey, Great Britain.

improved geometry, "light-piping" and a more efficient liquid scintillator the sensitivity of the method was enhanced. The method has now been giving satisfactory service for about 2 years.

EXPERIMENTAL

Apparatus and method

The basic design of the instrument is shown in Figs. 1 and 2. Developed paper chromatograms were cut into longitudinal strips, 2.5 cm wide, containing the labelled material. The strips were impregnated with liquid scintillator and mounted on the polished aluminium drum by threading the ends of the strip through a slot and clamping with the spiked and sprung clamp against the inside of the drum.

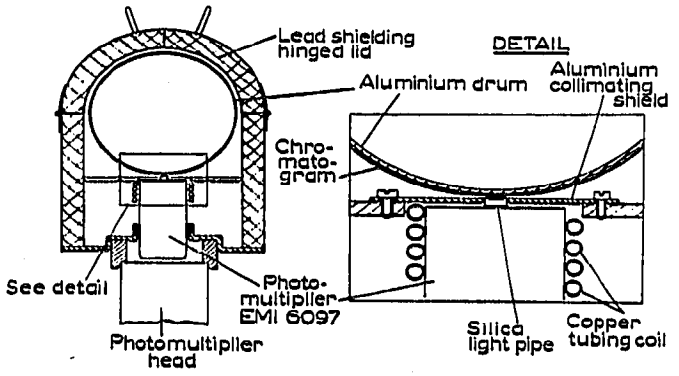

Fig. 1. Diagram of the instrument showing the method of assembly.

The light emitted by the scintillator passed from the chromatogram through a silica prism into the photo-cathode of the photomultiplier tube (E.M.I., type 6097). The silica prism ("Spectrosil" B-quality plate, made by the Thermal Syndicate Ltd., Wallsend, Northumberland, Great Britain), 26.00 mm × 8.00 mm × 3.90 mm, had its horizontal surfaces ground to a smooth finish and its vertical faces polished flat to $^1/_4$ fringe. It functions as an efficient "light pipe" and completely fills the collimating slit (8.0 mm wide). Optical contact between the silica prism and the photomultiplier tube was enhanced with a drop of silicone oil placed between them.

The signals from the photomultiplier tube passed through single channel electronics to a ratemeter and thence to a chart recorder.

The clockmotor drive of the scanner drum advanced the paper chromatogram past the collimating slit at the rate of 30 cm/h. The gear ratio of the chart drive of the recorder was selected to match this speed closely. No special effort was made, however, to synchronise the two movements absolutely.

The aluminium drum and photomultiplier assembly of the scanner were surrounded by a lead shield 5 cm thick. The photo-cathode was cooled by mains water passing through a coil of copper tubing surrounding the upper half of the photomultiplier tube. Although this heat exchanging device is not in direct contact with the photomultiplier tube, enough heat passes from the photo-cathode to the aluminium plate which contains the collimating slit and hence through the metal part of the scanner to the cooling coil, to reduce the background count to about 1.2 to 1.5 c.p.s.

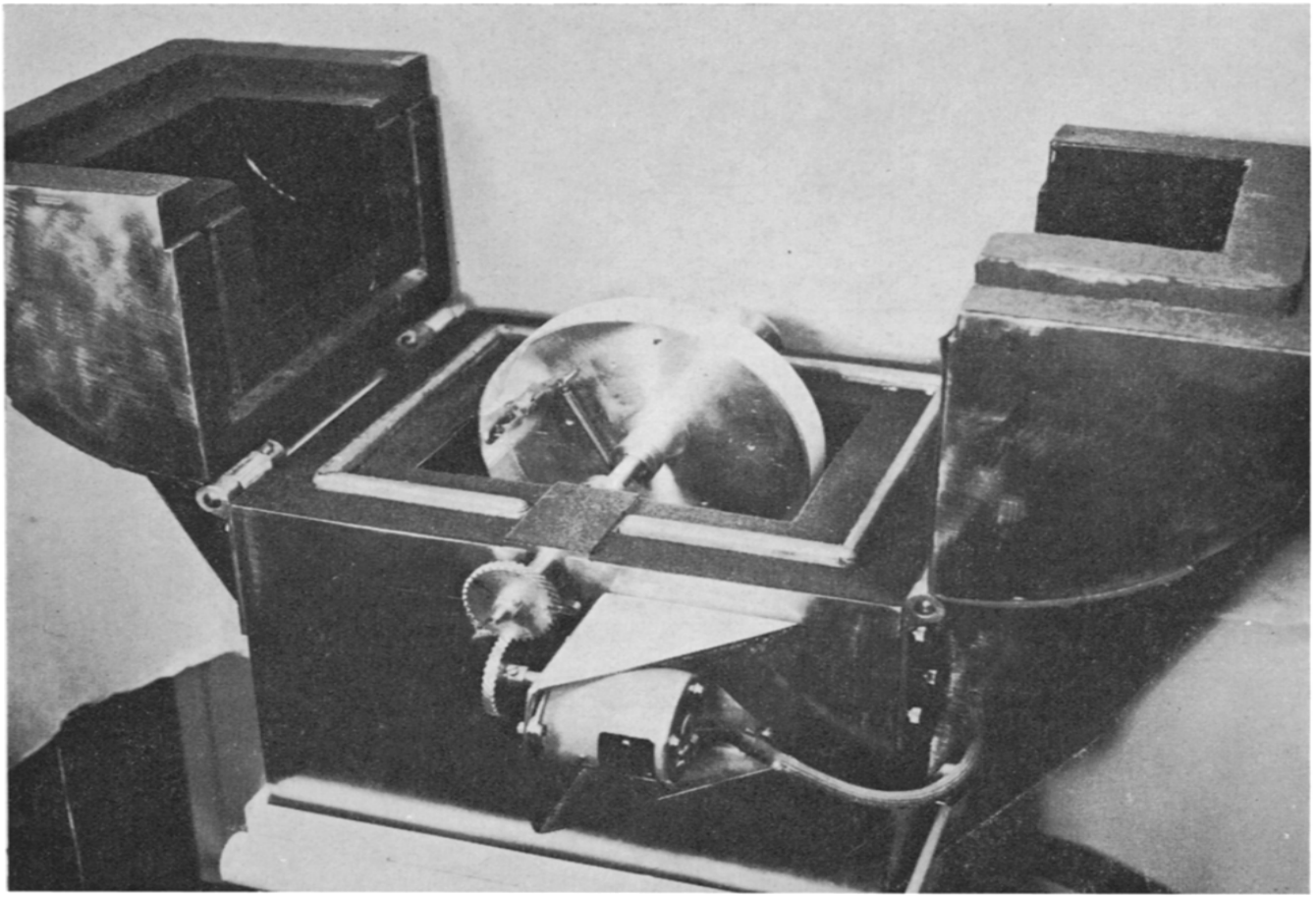

Fig. 2. View of instrument with open lids to show mounting of chromatograms.

This result was achieved with a mains water temperature of about +10°. No significant lowering of the background count rate was observed by pumping through the cooling coil water chilled to about +2°.

Materials

The liquid scintillator was purchased from Nuclear Enterprises (G.B.) Ltd., Edinburgh, Great Britain. D-Glucose-^{14}C (U) and D-ribose-^{14}C (U) were obtained from the Radiochemical Centre, Amersham, Buckinghamshire, Great Britain. Chromatography solvents were made up from laboratory grade materials which were not further purified prior to use. Whatman chromatography papers were used throughout.

Results

All figures stem from scans obtained on chromatograms developed for 24 h by the descending method. In no case has the scanned radioactive material been artificially confined to a conveniently small area on the paper.

The record of a scan (Fig. 3) of a chromatogram carrying five carboxyl-^{14}C labelled amino acids represents the performance of the technique near the limit of its sensitivity.

To check the linearity of the response for different levels of specific activity a

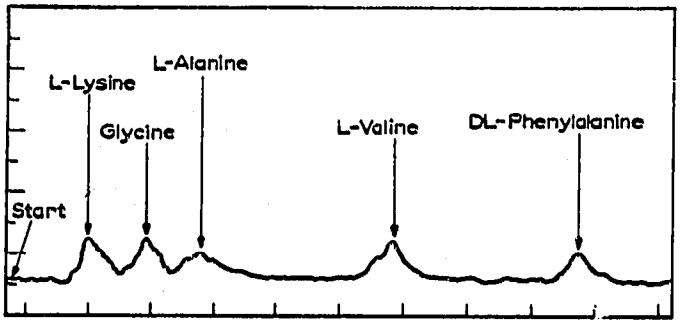

Fig. 3. Record of a chromatogram of 5 amino acids labelled in the carboxyl function with carbon-14. The chromatogram was developed on Whatman No. 3MM paper by descending *n*-butanol–acetic acid–water (4:1:1, v/v) for 24 h. Each amino acid (10 μg) was applied to the starting line in aqueous solution (10 μl) containing 0.1 mμC carbon-14 activity.

series of chromatograms was developed all containing D-glucose (10 μg) and D-ribose (10 μg). The weight (mg) of the peak area on the chart record of each chromatogram was taken as a direct measure of the response of the instrument to the amount of activity present in each "spot". The results are shown in Fig. 4.

Parallel chromatograms carrying unlabelled D-glucose (10 μg) and D-ribose (10 μg) were developed under identical conditions and sprayed with the silver nitrate reagent of TREVELYAN *et al.*[9] to determine the distance from the starting line

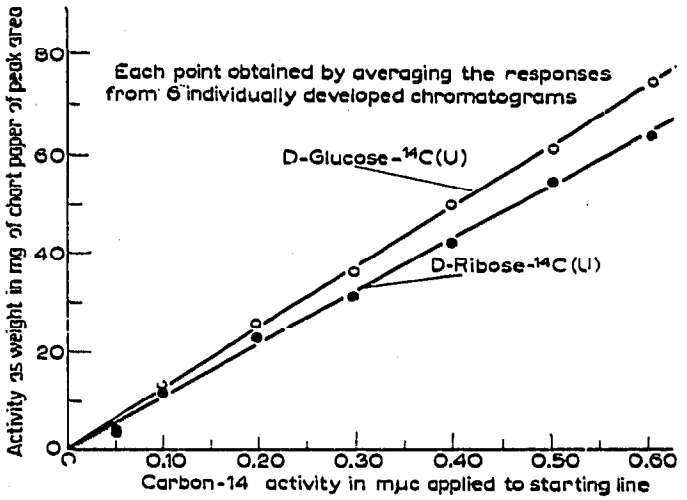

Fig. 4. Relationship between applied activity and response. The standard deviation of each "response" varied from $\pm$ 1.2 to $\pm$ 3.0 mg.

covered by the sugars and the area of the "spots" after 24 h' run when the chromatograms were removed from the tanks and scanned. In no case did the spots extend beyond the width of the strip (2.5 cm) cut from the chromatograms for scanning. D-Glucose travelled a distance of 8.1 cm and spread to cover an area of 6.5 cm^2. The analogous figures for D-ribose were 15.4 cm and 5.9 cm^2, respectively.

The results shown in Table I were produced to investigate the effect which thickness and texture of chromatography papers may have upon the response of the scanner to identical amounts of radio-activity. The peaks on the chart records were cut out and their weight taken as a measure of the response in the same manner in which the results shown in Fig. 4 were obtained.

TABLE I

EFFECT OF THICKNESS AND TEXTURE OF CHROMATOGRAPHY PAPERS UPON THE EFFICIENCY OF THE SCANNING METHOD

Chromatography papers	*Response to* D-*Glucose-*^{14}C *(U) (10 μg, 0.5 mμC). Weight of peak area ± standard deviation (mg)*
Whatman No. 1	50.1 ± 2.1
Whatman No. 2	49.9 ± 1.8
Whatman No. 3	54.2 ± 1.9
Whatman No. 3MM	53.8 ± 2.2
Whatman No. 54	46.6 ± 1.0

All chromatograms were developed for 24 h by descending *n*-butanol–acetic acid–water (4:1:1, v/v).

Each figure was obtained by averaging the response recorded on four identical paper chromatograms.

DISCUSSION

Earlier attempts made in this laboratory to find an efficient method of scintillator-scanning of radio paper chromatograms were based on the use of a solid, plastic scintillator. In this plastic material the scintillating compounds are dispersed in a matrix of polyvinyltoluene (NE 102 plastic scintillator, Nuclear Enterprises (G.B.) Ltd.). The geometry and the mechanical parts of the instrument were similar (Figs. 1 and 2), the plastic scintillator being machined to the same dimensions as the silica prism and occupying the same position in the collimating slit. The performance of this instrument was, however, inadequate. The prospects of enhancing the sensitivity of the instrument were improved with the introduction of a monoisopropylbiphenyl-based liquid scintillator. Compared with toluene or xylene, this solvent has a much lower volatility. It also confers upon the liquid scintillator a significant degree of immunity from quenching by dissolved oxygen (BUCK AND SWANK[10]). The latter property is of particular value in this work, since impregnated paper chromatograms present a relatively large surface area to air.

In paper chromatograms carrying compounds labelled with emitters of soft β-radiation a proportion of the electrons emitted by the decaying nuclides will become absorbed within the paper and will, therefore, fail to interact with any detector of radiation present outside the paper. The presence in the paper of the scintillator solvent in directly impregnated paper chromatograms largely attenuates this limitation (SELIGER AND AGRANOFF[3].) Furthermore, the organic solvent renders the paper relatively translucent (FUNT AND HETHERINGTON[4]).

The instrument was developed to its present state by replacing the originally used polyvinyltoluene "light pipe" with the silica prism in the collimating slit (Fig. 1).

Efficiency

It is difficult to give an absolute assessment of this parameter in paper chromatographic work. However, an idea of the efficiency of this technique may be gained from the results shown in Fig. 3. At the selected settings of the chart recorder full-

scale deflection corresponds to 10 d.p.s. (including background noise). The maximum net amplitude recorded in the amino acid peaks varies from 0.6–1.2 d.p.s. Since the amount of radio-activity of the ^{14}C label of each amino acid amounts to 3.7 d.p.s. (0.1 mμC), it follows that in scanning this particular chromatogram the instrument functioned with an efficiency of from 15 to 30 % at least. This degree of efficiency was maintained throughout and is of practical importance since after 24 h' developing 10 μg of any individual compound may spread to occupy an area of 5 cm^2 or more.

The results presented in Fig. 4 indicate that this method is capable of measuring the distribution of radio-activity in chromatograms with a satisfactory linearity of response. The lower slope recorded for the D-ribose-^{14}C (U) "spots" is due to carbon-14 labelled impurities present in the sample. This sample was about 2 years old at the time and the impurities may have resulted from radiolytic decomposition. A freeze-dried aliquot of an aqueous solution of this sample of carbon-14 labelled D-ribose was assayed in the "Packard" liquid scintillation spectrometer. The results showed that about 20 % of the nominal activity of this sample were volatile and were removed by freeze-drying. The difference between the two slopes in Fig. 4 suggests that the specific activity of D-ribose-^{14}C (U) used in the experiment was about 14 % below that of the D-glucose-^{14}C (U).

The results obtained on chromatograms carrying only 0.05 mμC carbon-14 in each sugar indicate that the efficiency of this method begins to falter under these conditions (area of glucose spot: 6.5 cm^2, area of ribose spot: 5.9 cm^2). The efficiency of the technique, even at these low activities could be improved by slower scanning, since all the scans reported above have been obtained at the relatively high scanning rate of 30 cm/h. A further improvement would result from subjecting the signals from the photomultiplier head to an upper as well as a lower discriminator limit.

Effect of the properties of chromatographic papers

The results in Table I show how the response to identical quantities of D-glucose-^{14}C (U) varies with papers of different thickness and texture. None of the results was significantly different ($P > 0.05$) from the response recorded on Whatman No. 1 paper. The practical usefulness of this method is borne out, however, by the response recorded on chromatograms run in the thick Whatman No. 3 and 3MM papers. In these, the response was slightly higher. It was significantly higher than the result obtained on Whatman No. 54 paper. This suggests that the efficiency of the technique depends upon the amount of liquid scintillator available in the immediate vicinity of the radio-active material in the chromatogram and thus tends to be enhanced, rather than impeded, in thick papers.

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SUMMARY

An instrument for the direct and continuous scanning of paper chromatograms

carrying soft-β emitters has been described. The method of detection is based upon a liquid scintillator with which the developed paper chromatograms are directly impregnated. The response is sufficiently linear to permit direct quantitative assay of radio-activity down to at least 0.1 mμC of carbon-14 per spot of 5 cm^2 or more surface area and is not attenuated in thick papers.

While retaining the advantages of liquid scintillation techniques in general, this method is free from the labour involved in cutting up developed paper chromatograms for counting in glass vials, nor is it subject to the error inherent in the variable geometry of orientation of the strips floating in the liquid scintillator.

The design of the mechanical parts of the scanner reduces to a minimum the amount of precision machining required in its manufacture. The required electronic circuits are those which in an increasing number of laboratories are becoming idle through the introduction of automated and transistorised liquid scintillation spectrometers.

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