THE USE OF A WINDOWLESS GAS-FLOW COUNTER FOR DETECTING WEAK β EMITTERS ON PAPER CHROMATOGRAMS

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INTRODUCTION

The windowless gas-flow scanner[1-10] is one of the most sensitive methods for detecting the distribution of weak β emitters on paper chromatogram strips. The fact that no window separates the counting volume from the active sample permits the estimation of the weakest β emitting isotopes, *i.e.* tritium, which has a maximum energy of 18 keV, all of the β particles being absorbed by windows with a superficial density greater than 0.6 mg/cm^2.[11] This type of detector also has the advantage that it produces a continuous trace of the distribution of activity on the chromatogram as opposed to the histogram produced by sub-dividing the paper into sections and counting these individually. These advantages would seem to indicate that a windowless flow counter is the ideal chromatogram scanner. The method does have its limitations, however, and when an apparatus of the type was set up in this laboratory to determine the distribution of tritium and carbon-14 labelled compounds on one-dimensional chromatograms the first results showed a complete lack of reproducibility. It was found that the discrepancies could be attributed to three basic causes, namely: the gas flow rate through the counter, the build up of static charge on the paper, and losses due to volatilisation of the active compound from the paper chromatogram. It was also found that scans from opposite sides of the same chromatogram were different, and that the scanner appears to be approximately eight times more sensitive to spots containing tritiated stearic acid than it is to spots containing tritiated benzoic acid on the same chromatogram. Each of these factors has been investigated separately and a procedure devised whereby it is possible to produce reproducible scans from the same chromatogram. The need to calibrate the sensitivity of the scanner for each individual spot on the chromatogram is discussed and a combustion procedure for this briefly described.

APPARATUS

The active chromatograms were scanned in a gas flow proportional counter, shown schematically in Fig. 1, which is similar in basic design to that described briefly by KISIELESKI AND SMETANA[6]. The paper chromatogram strip is attached to a plate that passes through a hemi-spherical counting chamber. A rack attached to the plate, driven by a pinion connected to an electric motor via a gear box, enables the speed
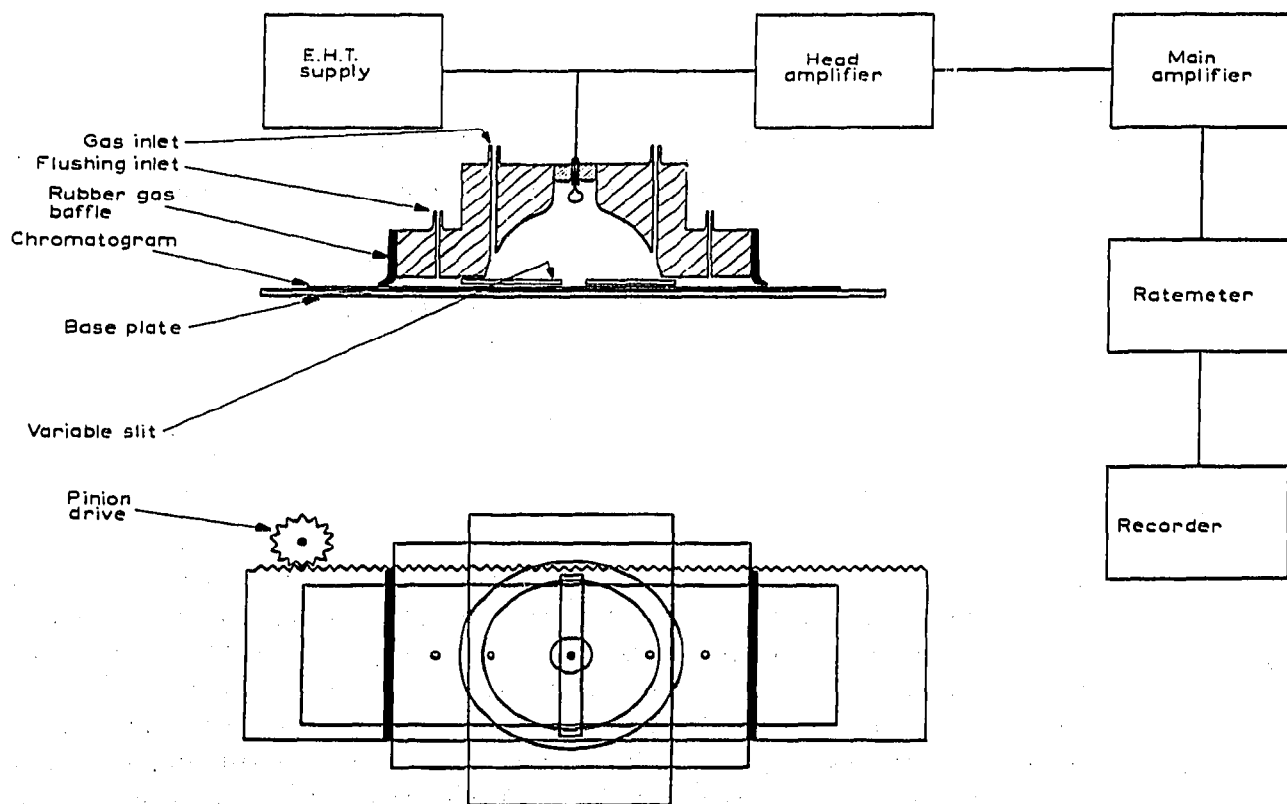
Fig. 1. A diagram showing the essential components of the chromatogram scanner and its associated electronic equipment.

at which the paper traverses the sensitive volume of the counter to be varied. A sliding plate containing rectangular slits of various dimensions is located between the counting chamber and the paper strip. This defines the area of the chromatogram counted at any one instant, and affects the resolution of spots. The counting gas (90 % argon, 10 % methane) is introduced at two points inside the hemi-spherical chamber, and at two points outside the sensitive volume. The gas inlets to the counting chamber are connected to a common supply which is regulated by a needle valve and measured with a Rotameter flow meter. The two flushing inlets are connected to a separate Rotameter. The gas passes out of the counter via the tunnels through which the plate and slit regulator pass.

The E.H.T. (about 2000 V) to the counter is supplied by a power unit (AERE Type 1359A). The pulses are amplified by a head amplifier (AERE Type 1049C), and a main amplifier (AERE Type 1430A), the output of which is connected to a ratemeter (AERE Type 1037C) and a Sunvic recorder.

EXPERIMENTAL

Setting up the equipment

To determine the optimum conditions for running the scanner a piece of filter paper spotted with tritiated stearic acid and carbon-14 labelled palmitic acid was sprayed with a 10 % polyethylene glycol 600 in ethyl alcohol solution, and allowed to dry. The paper was attached to the base plate and the appropriate spot introduced into the counter when necessary.

With the amplifier attenuation settings at 20 and 30 dB for tritium and carbon-14 respectively, and the bias on the ratemeter set at 50 V, the variation of count rate with E.H.T. was measured. The gas flow rates were 0.5 l/min and 0.05 l/min through the counting chamber and the preflushing tubes respectively. A point in the middle of the plateau thus obtained was selected as a suitable working voltage. To determine the optimum bias and attenuation settings the E.H.T. was set at this value and a series of curves of count rate (logarithmic scale) against bias voltage (linear scale) plotted for each attenuation setting of the amplifier. A similar series of curves were plotted for the background. The values of the ratio of count rate to background for the different bias and voltage settings were tabulated and the counter adjusted to the settings where this parameter was a maximum.

Gas flow rate

To investigate the effect of variations of gas flow on a stationary source one of the active spots on the test strip was positioned inside the counter. With the gas flow through the preflushing tubes fixed at 0.05 l/min the total gas flow through the central chamber was varied and the corresponding count rates recorded. Measurements for both carbon-14 and tritium were made for different discriminator and bias settings.

Static build up

The effect of the build up of static charge on the count rate of a stationary source, which has been reported by OSINSKI[8], was demonstrated by recording the variation, with time, of the count rate of spots containing tritium and carbon-14 on two test chromatograms. The electronic equipment was adjusted to the optimum settings for the two isotopes and the gas flow rates set at 0.5 and 0.05 l/min through the chamber and preflushing tubes respectively.

One of the chromatograms was then dipped in a 2 % solution of polyethylene glycol 600 in benzene and allowed to dry. The other chromatogram was sprayed with a suspension of colloidal graphite in aqueous ethyl alcohol until the chromatogram had a uniform dark grey colour.

After this treatment the change in count rate with time of the active areas on both chromatograms was again recorded.

Volatilisation of the sample

To determine the loss of activity during the preparation and storage of chromatograms a number of Whatman No. 1 papers were spotted with known volumes of freshly prepared solutions of tritiated benzoic and stearic acids in alcohols. Some strips were spotted with a single substance and others with a mixture of the two acids. The strips were run for approximately 7 h with a descending solvent consisting of ethyl alcohol (16 parts), 0.880 ammonia solution (1 part) and water (3 parts). The resultant chromatograms were dried and dipped rapidly in 2 % polyethylene glycol 600 in benzene. One chromatogram was rescanned after a second dipping to confirm that no loss of benzoic acid occurred at this stage.

The losses occurring between spotting a chromatogram and scanning it were determined by excising the active areas on chromatograms spotted with a single substance and run for 7 h. The paper was burned in a sealed flask of pure oxygen. A liquid phosphor containing inactive water was injected into the flask and an aliquot

removed after equilibration with the tritiated water formed during combustion[12]. A small piece of paper impregnated with the same volume of tritiated acid solution as that used to spot the chromatograms was assayed in a similar manner.

The chromatograms with two spots were marked with Indian ink containing non-volatile promethium-147[13] to give reference peaks of fixed height on the scans. One chromatogram was stored in the fume hood and scanned at regular intervals. The other was hermetically sealed in a polythene container, and rescanned after standing in the fume hood for 20 days. The polythene container was made from lay-flat tubing one end of which was sealed by placing the tubing between two metal plates and running a very small gas flame over the polythene where it passed through the plates. The chromatogram was inserted in the bag thus formed, and the open end flame sealed when all of the air had been expelled.

Distribution of activity within the chromatogram

A strip of Whatman No. 1 paper 4 cm × 46 cm was spotted with 5 μl of a freshly prepared alcoholic solution of benzoic acid (1.75 μg containing 2.52 μC of tritium) and stearic acid (2.45 μg containing 1.48 μC). Both samples were applied to the same side of the paper by repeatedly touching the surface with a capillary tube containing the active solution. The evaporation of the solvent was accelerated by applying a draught of air over both surfaces of the paper. The paper was equilibrated for 5 h in an atmosphere saturated with the solvent, ethyl alcohol (16 parts) 0.880 ammonia solution (1 part), and water (3 parts), and run for 10 h using the descending solvent technique. The chromatogram was dried, dipped in 2 % polyethylene glycol in benzene, and each face was scanned twice. The areas of paper containing the active spots were then cut from the chromatogram and the activity contained in them determined after combustion[12].

RESULTS AND DISCUSSION

It was found that tritium required a higher gain (regulated by the attenuation setting of the main amplifier) than carbon-14 at optimum counting conditions. This is to be expected because of the difference in their average energies which are 5.5 keV and 50.0 keV for tritium and carbon-14 respectively[11].

Gas flow rate

The effect of gas flow rate on the variation of the count rate of spots containing tritium and carbon-14 on the same chromatogram with high gain and a low bias setting is shown in Fig. 2. Reducing the gain and increasing the bias reduces the counting efficiency for both isotopes, and higher gas flows are required for the count rates to reach a steady value, as shown in Fig. 3.

Back diffusion of air into the counting chamber reduces the gas amplification, this reduces the size of the pulses and results in a loss of counts. This can be averted by increasing the gas flow. However, since a higher gas flow rate increases both the build up' of static charge, and the rate of loss of volatile compounds from the paper, it should be kept to a minimum. This can be achieved by working with high gain and low discriminator settings, and by designing the equipment in such a manner that the possibility of back diffusion of air into the counting chamber is reduced to a minimum.
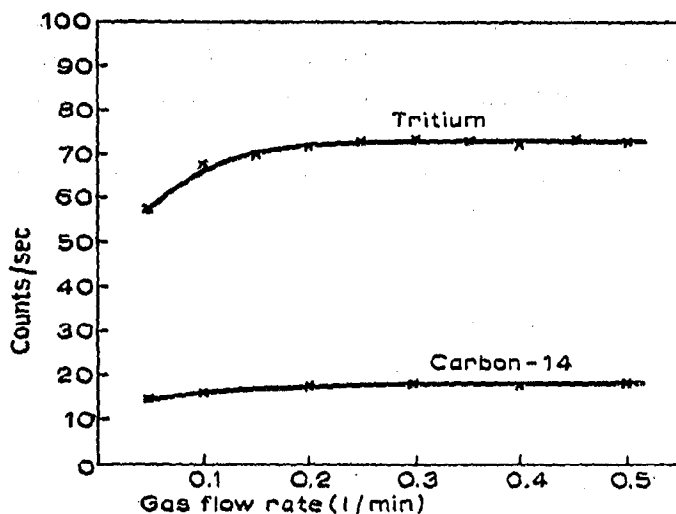
Fig. 2. A graph showing the variation of count rate with the total gas flow through the centra counting chamber with the attenuation on the amplifier set at 8 dB and a bias voltage of 15 V The total gas flow through the flushing tubes was maintained at 0.05 l/min throughout.

Static build up

The rate of loss of counts on a stationary chromatogram due to the build up of static charge is shown in Fig. 4. It can be seen that the losses for tritium are far greater than those for carbon-14. The major factors affecting the rate of decrease of counts were found to be the gas flow rate and the slit width, the losses increasing with increasing gas flow and decreasing when the slit width was decreased.

It was found that when untreated chromatograms containing a number of spots of non-volatile tritiated compounds were scanned on successive days the peak heights varied considerably, reaching a maximum when atmospheric humidity was high. If the same chromatograms were repeatedly scanned, without allowing the paper to
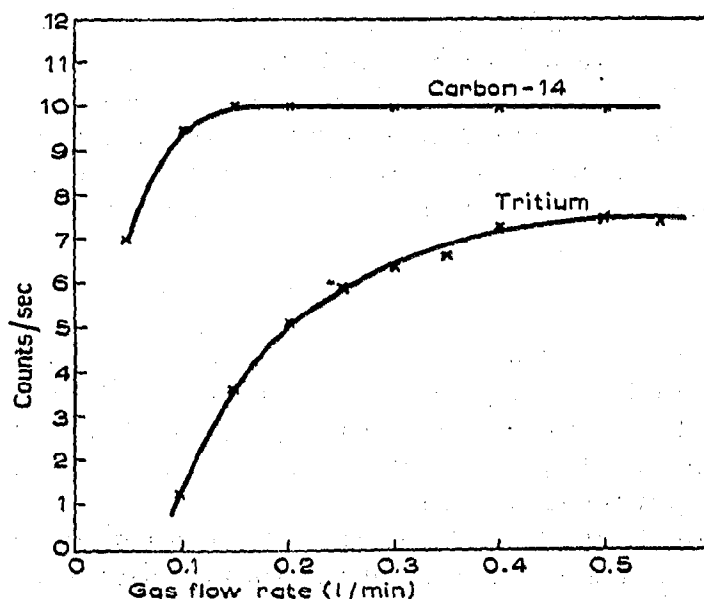

Fig. 3. The variation of the count rate with gas flow rate for the same chromatogram spots used for Fig. 2, but with the attenuation and bias settings adjusted to 16 dB and 50 V respectively.

equilibrate with the atmosphere between scans, the tritium peaks were successively reduced. Successive scans were more reproducible if the counting gas was partially saturated with water vapour but this resulted in an overall lowering of the peak heights.

The problem of the build up of static charge can be completely overcome by rendering the papers more conducting. Two methods have been devised and tested;
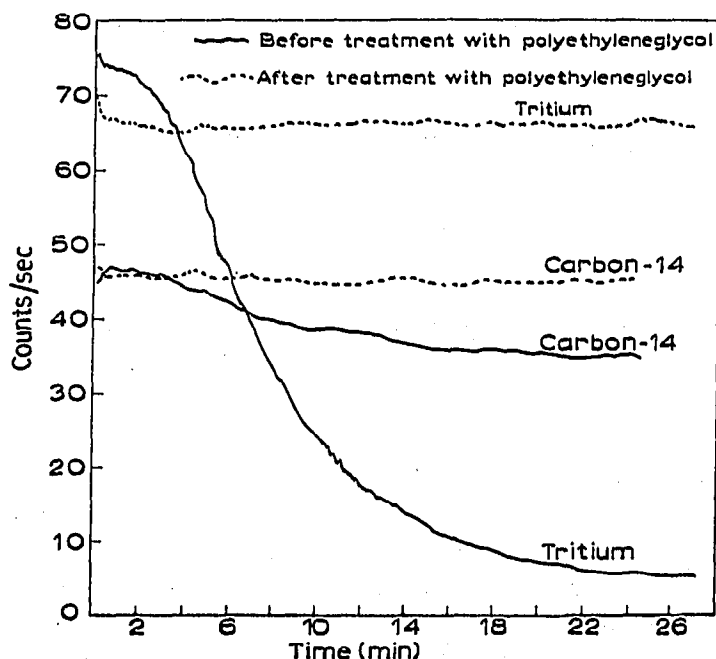

Fig. 4. The variation of the count rate with time of spots containing tritium and carbon-14 on a stationary chromatogram.

one consists of spraying the paper with colloidal graphite, the other consists of dipping or spraying the paper with a solution of a humectant—a substance that keeps the moisture content constant despite environmental changes. Polyethylene glycol 600 was found to be suitable for this purpose. The effect of the latter treatment, which is to be preferred if spots are also to be detected with a colour reagent, is shown in Fig. 4. The peak heights produced by spots containing tritium and carbon-14 on papers treated by either of these methods do not vary by more than 2 % on repeated successive scans.

Loss of activity

The mass of a radioactive material of high specific activity that can be detected by its radiations is very small, usually far less than can be detected by other means. When very small weights of compounds, normally regarded as stable and non volatile, are spread over a relatively large area of paper the fractional losses due to a quite small vapour pressure can be appreciable.

The losses occurring during the preparation of the chromatograms were measured by a combustion method. The count rate of the spots containing approximately 2 μg of benzoic and 2 μg of stearic acid on the final chromatogram was found to be approximately 10 % less than that of the solution applied to the paper for both acids. A
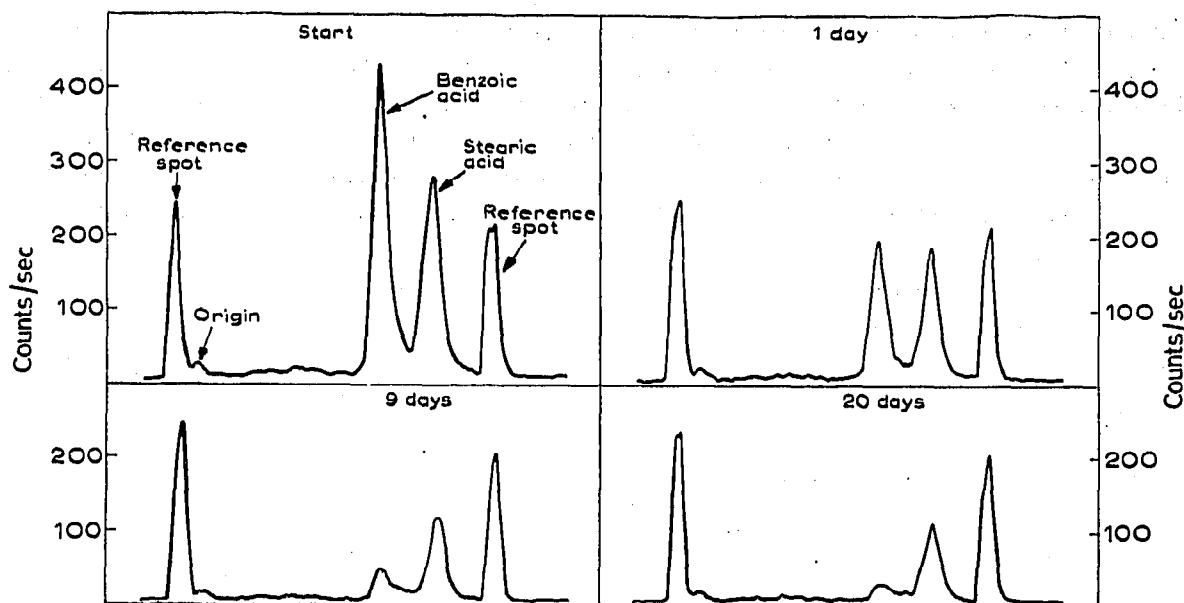
Fig. 5. The variation of scans obtained from the same chromatogram after exposing it to a draught in a fume cupboard for a number of days.

portion of these losses can be attributed to the separation of radiochemical impurities during the chromatographic process.

The change in peak heights that occurs when the chromatogram is exposed to a draught of air over a period of 20 days is shown in Fig. 5. The relatively small reductions in peak height shown in Fig. 6 indicate that these losses can be considerably reduced by encasing the chromatogram in polythene.

Distribution of activity within the chromatogram

The traces obtained when different surfaces of the same chromatogram are scanned are shown in Fig. 7. It can be seen that the peaks on the surface opposite that to which the solutions were applied are greater for both acids. A possible explanation for this effect is that a higher concentration of acids is built up on the side of the paper opposite that to which the capillary is applied during the spotting of the paper. Each time the capillary touches the paper it releases a drop of solution which washes
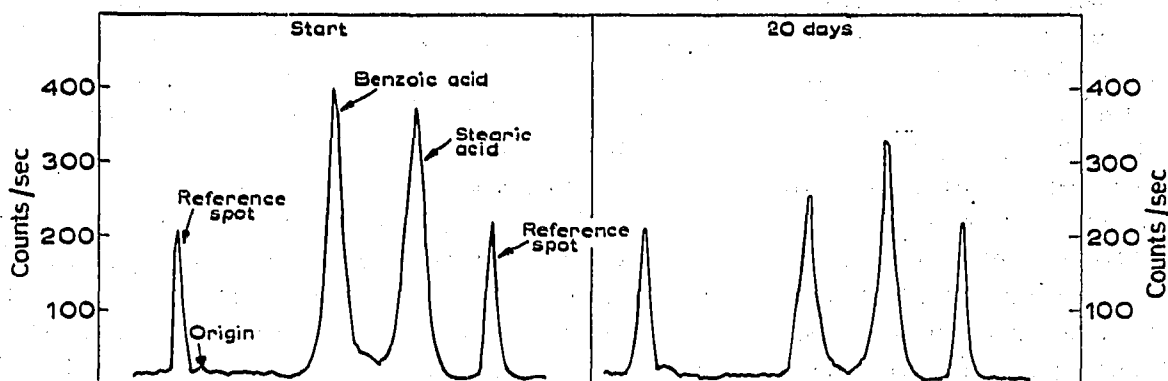

Fig. 6. The variation of scans obtained from a chromatogram containing the same amount of activity as that used for Fig. 5 sealed in a polythene tube from which air was excluded for 20 days.

the acid already in the paper before it as it passes through the paper. What is surprising is that a concentration gradient should be maintained through the relatively small thickness of the paper during the 10 h for which the eluting solvent is passing down the paper. These results, which have been repeatedly reproduced, are in contrast to those of POCCHIARI AND ROSSI[14,15] who observed that different concentrations of activity were detectable on opposite sides of the paper when Whatman No. 1
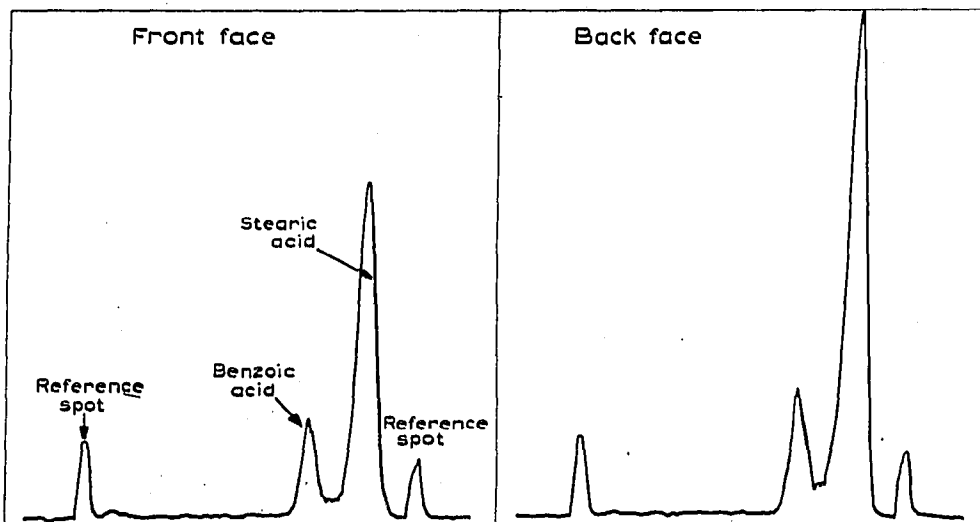

Fig. 7. Scans obtained from different sides of the same chromatograms. The face denoted FRONT was the face to which the spot was applied.

papers were spotted with carbon-14 labelled glucose. They found that chromatographic development led to homogeneous distribution of the activity throughout the paper except for substances left at the origin. WENZEL[10], however, found that the count rate on the side of the paper to which the solution was applied was greater than the reverse side, and that a difference in count rate for the two sides of the paper was maintained after chromatography. HANSEN[16] has observed a small error when scanning both sides of a chromatogram for carbon-14 labelled stearic and palmitic acids, but he does not indicate if the discrepancies are related to the side of the paper to which the original solutions were applied. BIDWELL[17] has observed that there is an erratic increase in surface concentration of carbon-14 labelled compounds when papers are forcibly dried on one face. He used Whatman No. 3 MM paper which is considerably thicker than the Whatman No. 1 papers, used for the experiments described herein, which were usually dried in a gentle draft—the process only taking a few minutes. There was no noticeable difference in the author's results after the papers were forcibly dried on one surface.

A more surprising fact was revealed by combustion of the spots that produced the peaks shown in Fig. 7. The assay showed that the area of the chromatogram corresponding to the benzoic acid peak contained 2.23 μC of the original 2.52 μC of tritium originally applied, and the stearic acid peak contained 1.24 μC of the original 1.48 μC. The area of the stearic acid peak was found to be 4.05 times greater than that of the benzoic acid peak for the front of the chromatogram and 4.55 times for the back. Thus for this pair of acids the normal premise that peak areas are proportional to the activity of the corresponding spots, for the same isotopes on a single chromato-

gram, is invalidated by a factor of eight. A possible explanation is that the benzoic acid is more volatile than the stearic acid and therefore the surface concentration is less than the bulk concentration due to the differences in their vapour pressures. The half-thickness for absorption of tritium β particles is about 0.1 mg/cm² and all are absorbed when passing through substances of density greater than 0.6 mg/cm². Therefore only tritiated compounds on the outside of the outermost fibres of the paper are detectable. An alternative and more plausible explanation is that the effect is the result of an inherent fundamental process in paper chromatography, the benzoic acid being retained within the cellulose fibres of the paper whereas the stearic acid remains primarily on the surface.

If the second of these explanations is correct the effect could have more far-reaching consequences. For example, the results obtained by the colorimetric determination of concentrations based on surface reactions may be subject to error if the concentration of the substances investigated not only varies from one surface of the paper chromatogram to the other, but also within the cellulose fibres of which the paper is composed.

RECOMMENDED PROCEDURE

In order to obtain reproducible scans from chromatograms containing substances labelled with tritium and carbon-14 the following procedure is recommended: firstly, having found the optimum settings for the electronic equipment for the particular isotope to be measured, the minimum gas flow rate to give a steady count for a sample should be found. Increasing the flow by 50 % of this value will normally compensate for additional diffusion when the chromatogram is mobile. Spraying the chromatogram with colloidal graphite to a uniform dark grey colour will prevent the build up of static charge on the paper, which has a pronounced effect when scanning for tritiated substances. Alternatively, the papers can be dipped or sprayed with polyethylene glycol solution. There is a possibility that opposite faces of the same chromatogram will give different scans. It is important therefore that the same side of the chromatogram, with respect to that to which the spot was applied, should always be kept uppermost in the chamber if repeated scans are to be made. If the scans are to be used as the basis for quantitative measurements both faces should be scanned and the results averaged. Chromatograms containing small masses of substances with small vapour pressures should be scanned as soon as possible after preparation. The loss of activity from papers that have to be kept before scanning, or stored for future use, can be considerably reduced if they are hermetically sealed in a polythene tube from which the air has been excluded.

Although adsorption of the above procedure should result in the production of reproducible scans from a single chromatogram containing tritium, it should not be automatically assumed that the same factor can be used to convert peak heights or areas to activity for all compounds. For accurate quantitative analyses the activity of each spot should be checked independently, *i.e.* by combustion.

SUMMARY

A 2 π gas flow proportional counter for scanning chromatograms is described. The effects of the gas flow rate, build up of static charge, and the volatilisation of the

sample on the reproducibility of scans are discussed and methods whereby artefacts due to these causes can be minimised are outlined. It is shown that different scans can be obtained from opposite faces of the same chromatogram. It is also shown that when using tritium the peak height, or peak area, on a scan does not necessarily correspond with the activity of the spot on the chromatogram for different substances. Thus a spot containing 1.2 μC of tritiated stearic acid produces a peak on the scan with an area four times greater than that produced by a spot containing 2.2 μC of tritiated benzoic acid on the same chromatogram. An operational procedure based on these findings is outlined.

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