CHROM. 3502

Quantitative scanning of ^{125}I on thin-layer chromatograms

The techniques of locating and quantitating ^{125}I-labelled compounds on thin-layer chromatograms have, hitherto, been based on autoradiography[1,2] or on liquid or solid phosphor[3] scintillation counting of eluates or zonal scrapings from the plate. Although scanning would offer many advantages, the 27keV X-rays emitted by the isotope would be expected to be detected with low efficiency by the ambient pressure gas flow Geiger-Müller and proportional detectors of commercially available chromatogram radio-scanners.

The present communication describes a high-efficiency, low-background scanner for counting ^{125}I on thin-layer plates.

Apparatus

The apparatus (Fig. 1) consists of a thallium-activated sodium iodide crystal, in disc form (25 mm diameter × 1 mm; Nuclear Enterprises, Great Britain) fitted with a beryllium end window and optically coupled by silicone oil to the window of a photomultiplier tube (E.M.I. type 6097B). This photomultiplier–crystal assembly is mounted in a standard support tube* closed at one end by a cap machined to form a focussing rectangular collimator of external dimensions 18 × 1 mm. Access of stray light into the detector is prevented by interposing two sheets of aluminized Melinex foil (George Whiley Ltd.) between the crystal and the end cap. The whole detector assembly is shielded from background radiation by a lead castle (wall thickness 5 cm).

In an alternative form of detector which was examined, the disc crystal is replaced by a wedge-shaped thallium-activated caesium iodide crystal which fits directly into the collimated slit. The upper face of the crystal is cemented to a glass disc, 3.5 cm in diameter, which is optically coupled to the photomultiplier.

Signals from the photomultiplier are fed via an emitter follower* and amplifier/pulse height analyser module* to a ratemeter* driving a modified chart recorder. In applications where quantitation of the radioactive peaks is required, a second output signal from the amplifier/pulse height analyser is fed to a scaler* which may be interrogated at pre-determined time intervals by a fast parallel printer (Kienzle, type KN66) and ancillary printer control unit*.

A horizontal extension platform covered with black "vitrolite" is bolted to the recording milliammeter (Texas Instruments, model 8415) and the chart storage spool is transferred from the recorder to the end of the platform. The chart paper is fed from this spool, across the platform and under a conveniently located roller to the drive sprockets of the recorder; a bridge across the platform supports the photomultiplier assembly and its lead shielding. Transport of thin-layer chromatograms under the collimated slit of the detector is accomplished, without the need for an additional, synchronised drive gear box assembly, merely by resting the thin-layer plates on the horizontally extended chart paper.

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* Panax Equipment Ltd. It is understood that this company propose to market a thin-layer scintillation scanner embodying these principles.

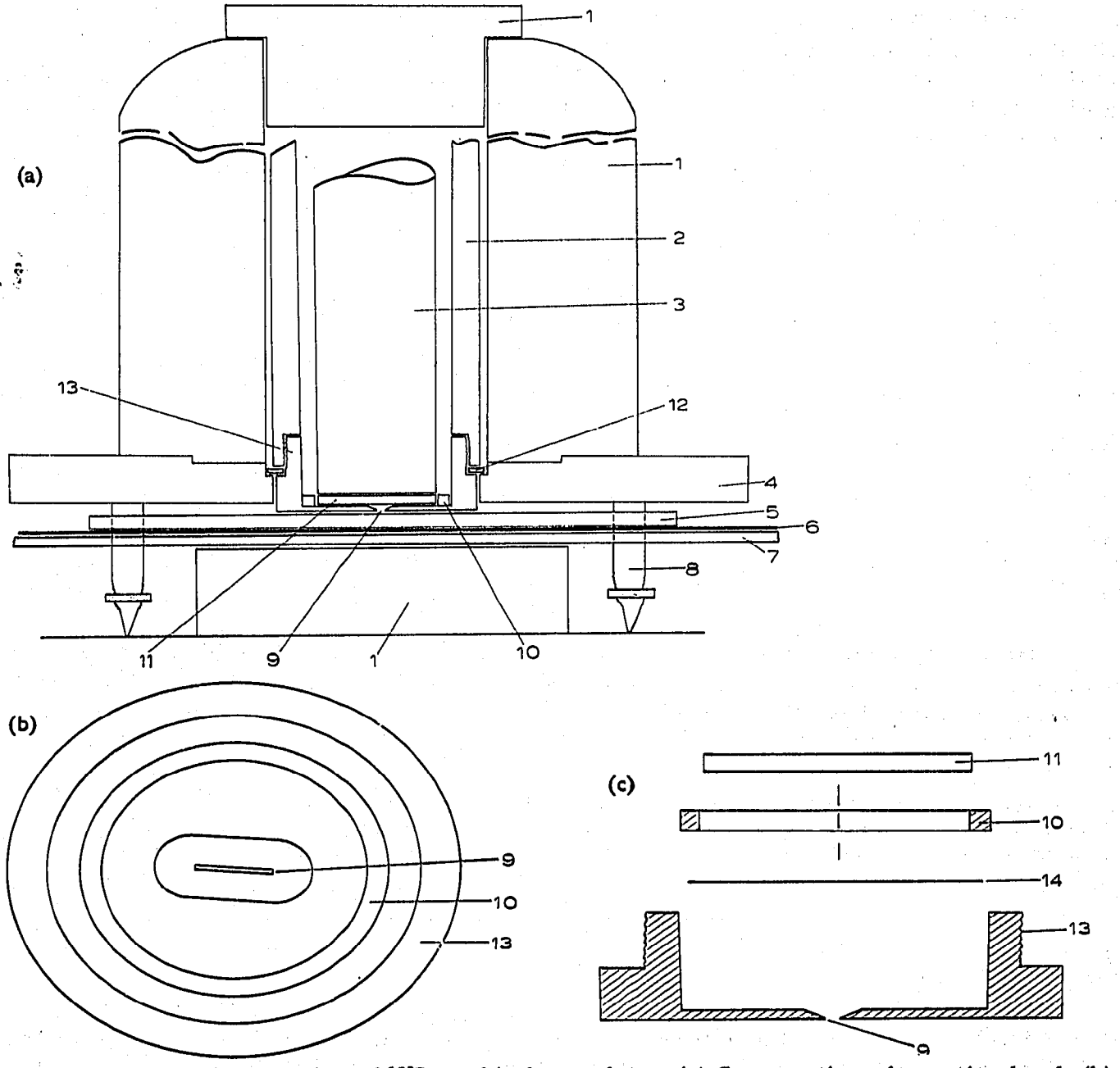

Fig. 1. Apparatus for scanning of ^{125}I on thin-layer plates. (a) Cross section of counting head. (b) Plan showing detail at 9. (c) Elevation showing detail at 9. 1 = Lead shielding; 2 = photomultiplier housing; 3 = photomultiplier; 4 = support bridge; 5 = thin-layer plate; 6 = chart paper; 7 = vitrolite-covered extension to recorder; 8 = height adjustment; 9 = slit; 10 = crystal spacer ring; 11 = crystal; 12 = support ring; 13 = end cap; 14 = aluminised Melinex.

Results

The efficiency of the counter was determined by applying known quantities of ^{125}I to thin-layer plates (starch bound Kieselgel N, 0.25 mm thick, on 4 mm thick glass) and scanning the chromatograms using both the ratemeter and scaler-printer presentations. The counting efficiency, η, calculated from the expression $\eta = 10^2 nv/Nb$, where n = observed gross counts, N = applied activity (d.p.s.), v = scan speed (mm/sec) and b = slit width (mm), was found to be 54 %.

Further work, summarised in Table I, showed that the counting efficiency was substantially independent of variations in the vertical clearance between the layer and the collimator slit. In the contrast, the peak height displayed on the ratemeter and recorder was found to be dependent on this operational parameter. Table I shows the observed changes in the peak ratemeter reading (c.p.s.), expressed as a percentage of the applied activity (d.p.s.), for typical chromatogram spots. The declining peak height with increasing clearance was accompanied by a noticeable broadening of the peaks.

TABLE I

DETERMINATION OF THE EFFICIENCY OF THE THIN-LAYER SCANNER

Plate-collimator clearance (*mm*)	*Counting efficiency* (%)	*Peak reading/applied activity* (%)
0.4	54.5	15.4
1.4	53	12.5
2.9	50	9.6
3.95	51.7	7.8

In order to determine the reproducibility of determinations with the thin-layer scanner, replicate scans of $^{125}I^-$ on chromatograms were carried out, results of which are summarised in Table II.

TABLE II

REPLICATE SCANNING OF $^{125}I^-$ ON CHROMATOGRAMS

Applied activity (*d.p.s.*)	*No. of scans*	*Mean observed activity* (*c.p.s.*)	*Observed S.D.* (%)	*Calculated S.D.* (%)
740	12	392	3.2	3.4

The resolution of the counter was next examined. $^{125}I^-$ was applied to a chromatogram as spots, 1.5 mm in diameter, spaced at 2.5, 5 and 10 mm between centres and leaving, therefore, intervening blank zones of 1, 3.5 and 8.5 mm. Scanning of the chromatogram using appropriate ratemeter settings indicated that the 5 and 10 mm peaks were fully separated and the spots at 2.5 mm between centres were partially resolved (ratemeter reading at centre approximately 60 % of maximum).

The background count rate of the detector, with the pulse height analyser set to provide a 27.5 mV "window" was 11.6 $\pm$ 0.3 c.p.m. With only the lower discriminator in use, the "open window" background count rate was 45 $\pm$ 0.6 c.p.m.

A typical scan, obtained with this equipment, of a thin-layer chromatogram of the labelled iodoamino acids of plaice is shown in Fig. 2.

Similar experiments on the wedge-shaped thallium-activated caesium iodide crystal showed this to be inferior in performance. The counting efficiency was 34 %,

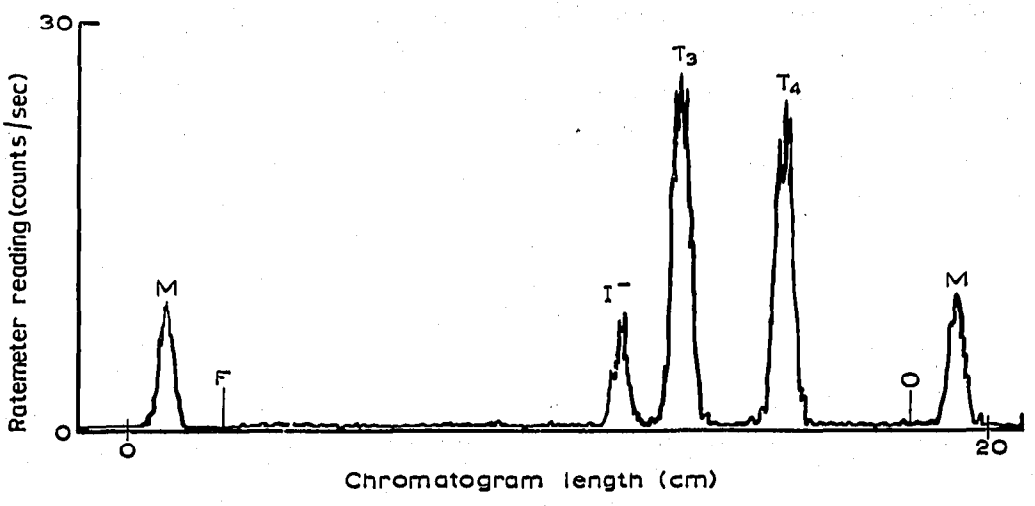

Fig. 2. Scan of ^{125}I labelled iodoamino acids from plaice plasma. Scan speed, 6 in./h. M = Marker; O = origin; F = solvent front; I^- = iodide; T_3 = 3,5,3′-triiodothyronine; T_4 = thyroxine. System: chloroform–methanol–0.880 ammonia (50:25:2.5).

while the background count rate (25 mV window) was 11.3 ± 0.3 c.p.m. The "open window" background count rate was 18.6 ± 0.5 c.p.m. and the count rate outside the lead castle was 26 c.p.m.

Discussion

Autoradiography is perhaps the most commonly used method of detecting ^{125}I-labelled compounds on thin-layer chromatograms[1]. It is, however, excessively time consuming, particularly when multiple exposures are necessary to determine, by densitometry, minor and major radioactive components on the same chromatogram. During this time, serious decomposition of sensitive materials may occur; ^{125}I thyroxine for example has been found (OSBORN AND SIMPSON, unpublished observations) to suffer objectionable degradation during autoradiography at 0° for three days. Solid scintillation counting of scrapings or eluates from thin-layer plates should be regarded as a method assaying known compounds, while SNYDER AND KIMBLE's technique[4] of counting zonal scrapings from chromatograms in liquid scintillation mixtures is not only costly in time and materials, but makes recovery of the radioactive compounds difficult. Although it is possible to detect ^{125}I-labelled compounds on thin-layer plates by commercially available gas flow Geiger-Müller or proportional counter scanners, it was found, as expected, that counting efficiencies were only of the order of 6% (OSBORN AND SIMPSON, unpublished results).

The design of the present apparatus was dictated by the need to determine rapidly and accurately the profiles of very low levels of ^{125}I activity on thin-layer chromatograms. The scanner described above has been in constant use for twelve months and has consistently shown counting efficiencies for ^{125}I of approximately 54%.

The maximum theoretical efficiency for a counter whose collimator has an included angle of 145° is 40%; the higher observed efficiency suggests a significant X-ray transmission near the edges of the collimator slit but must result, also, from the fact that at finite clearances between the collimator and the thin-layer plate, the scintillator crystal is able to "see" beyond the limits of the slit width. This is confirmed

by the fact that the resolution obtained with the instrument is rather poorer than would be expected if the slit width were truly 1 mm.

The standard deviation of determinations of radioactive emission by scanning methods is necessarily higher than when the whole radioactive material is counted at one time. This follows from the expression:

$$d^2 = \frac{10^4 K^2 t(\sqrt{r_1} + \sqrt{r_2} + \sqrt{r_3} \cdots\cdots)^2}{n^2} \qquad (1)$$

where

d = deviation (%)
K = confidence constant
t = slit residence time (slit width ÷ scan speed)
r_1, r_2, r_3 etc. = mean count rates in successive slit width sections of the spot
n = observed gross counts.

which, under scanning conditions, replaces the familiar expression $d^2 = 10^4 K^2/n$, defining gross counting statistics. The close agreement between the observed standard deviation ($K = 1$) of counts determined on replicate chromatograms and the standard deviation, calculated according to eqn. (1) from the count rates determined at intervals during the scanning of a single chromatogram peak, suggests that errors resulting from differences in the X-ray absorption and back scatter of different plates may normally be ignored.

The experiments with the wedge-shaped thallium-activated caesium iodide crystal were carried out in the hope of achieving lower backgrounds (by reducing the crystal volume) and hence a higher "figure of merit", E^2/B; this figure determines the confidence with which low levels of activity may be detected. Although backgrounds were not significantly lowered, possibly due to an inherently lower pulse resolution in a crystal of this geometry and to an increase in photomultiplier noise at the necessary higher tube potential, such crystals may find useful application where, for any reason, pulse height analysis is not practicable or the amount of shielding available is limited.

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