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Note

The application of a thermionic detector to the determination of nitrogen-containing compounds on thin-layer chromatograms

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For the analysis of various biologically active compounds, including antibiotics, benzimidazole anthelmintics, and carbamate pesticides, in animal tissues, a sensitive and specific detector for nitrogen-containing compounds on thin-layer chromatograms was required. Many of these compounds are not readily amenable to analysis by gas chromatography (GC), either because they are insufficiently volatile *per se* and therefore require laborious and time-consuming derivatization procedures; or because they are too labile to withstand the high temperatures necessary for chromatography in the gas phase. However, thin-layer chromatography (TLC) systems have been devised for all of the compounds of interest using techniques such as bioautography[1], fluorimetry[1] and colorimetry[1] to locate and quantify compounds on chromatograms.

GC flame ionisation detectors have been adapted for the quantitation of thin-layer chromatograms[2–4]. This technique represents a considerable advance in the TLC analysis of compounds that cannot easily be made visible with spray reagents, but it suffers the disadvantages of being relatively insensitive and non-selective. To overcome these disadvantages a GC thermionic ionisation detector (TID) was adapted to the quantitative evaluation of thin-layer chromatograms.

This paper describes the construction and application of a TID to the evaluation on thin-layer chromatograms of two carbamate pesticides, bendiocarb (2,2-dimethyl-1,3-benzodioxol-4-yl N-methylcarbamate) and dimetilan (2-dimethyl carbamoyl-3-methyl-5-pyrazolyl dimethylcarbamate). The possible application of the instrument to measurements of benzimidazole anthelmintics is discussed.

EXPERIMENTAL AND RESULTS

Apparatus

The basic instrument is essentially similar to that described by Padley[3] except that the tension bar was not detachable from the instrument. The tension bar could be driven at speeds ranging from 4 to 40 cm/min by an electric motor fitted with a variable speed gearbox.

The detector was a Pye Panchromatograph (Pye-Unicam, Cambridge, Great Britain) FID modified to the configuration shown in Fig. 1. Pure hydrogen was fed

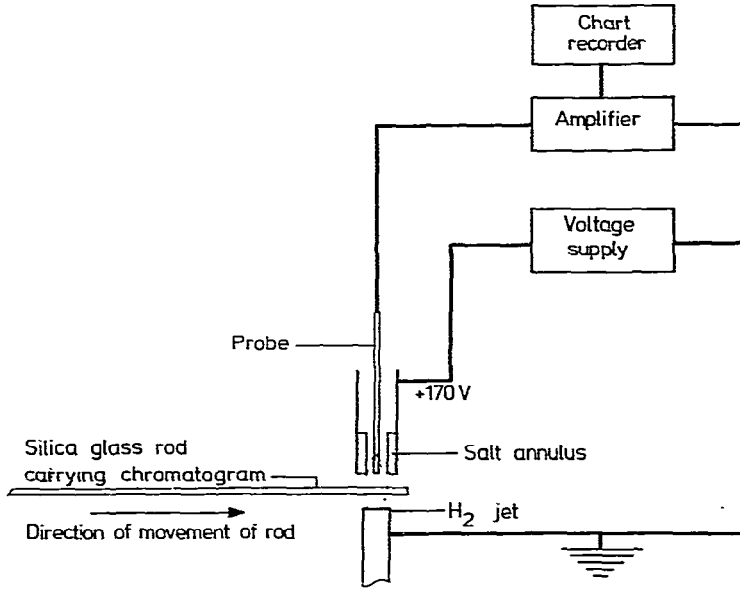


Fig. 1. Schematic diagram showing configuration of detection system.

to the detector jet and the flame was allowed to burn in air. The rubidium chloride salt tip was from a standard Pye-Unicam Series 104 GC nitrogen detector and the amplifier and voltage supply were from the same instrument. The chromatograms were recorded and the areas under peaks were integrated using a 1-mV Model 225 M Pantos Unicorder fitted with a Model OP-041 integrator (Nippon Denshi Kagaku, Kyoto, Japan).

Thin-layer chromatography

Silica glass rods 0.9 mm diameter × 150 mm long were coated with either aluminium oxide or silica gel G (Merck, Darmstadt, G.F.R.) as described by Padley[3]. The coated rod was allowed to dry in air and then passed slowly through the flame of a bunsen burner. The mixture to be separated was then placed on the rod approximately 2 cm from one end. The rod was placed vertically in a 2.5 × 20 cm test-tube containing approximately 1 cm depth of developing solvent and lined with filter paper to maintain an atmosphere saturated with solvent vapour. The tube was stoppered and the chromatogram allowed to develop to a distance of about 10 cm from the origin.

Detection

The rod was removed, dried in air and placed in the flame of the instrument with the starting point of the chromatogram just outside the detector. The nozzle, salt tip, collector electrode, flame size and other parameters were adjusted (Table I) and the compounds were then detected by passing the rod through the flame in the direction of development of the chromatogram.

TABLE I

TYPICAL CONDITIONS USED FOR DETECTING BENDIOCARB AND DIMETILAN ON A CHROMATOGRAM

Parameter	*Value*
Rod diameter	0.9 mm
Thickness of coating	0.1 mm
Distance between nozzle and salt tip	1.5 mm
Distance between nozzle and lower edge of rod	0.25 mm
Hydrogen flow	40 ml/min
Polarizing voltage	170 V
Standing current	10^{-10} A
Rod speed	10 mm/min
Recorder chart speed	40 mm/min
Amplifier attenuation	10×10^2 (equivalent to 10^{-9} A full scale deflection)

It was found necessary to vary the rod speed according to the compound being measured. With compounds of comparatively low volatility it was necessary to use a low speed to ensure complete volatilisation of the compound with one pass through the detector. However, because the baseline noise level decreased with increasing speed, the rod speed was selected to give the best possible baseline consistent with complete volatilisation of the compound(s).

A standing current of the order of 10^{-10} A was found to give the best signal-to-noise ratio.

Reproducibility

To test the reproducibility of detector response 16 chromatograms of a mixture containing 0.5 μg each of bendiocarb and dimetilan were run successively on the same rod using hexane–acetone (1:1, v/v) as developing solvent. Immediately after scanning each chromatogram and with the rod still *in situ* in the scanner, 1 μl of a standard solution containing 0.5 μg of either bendiocarb or dimetilan was placed on the rod with a micropipette at the position occupied by the compound on the chromatogram. The rod was then scanned a second time and the areas of the peaks produced by these "spotted" standards (Fig. 2b) were compared with the areas of peaks produced by chromatographed standards (Fig. 2a).

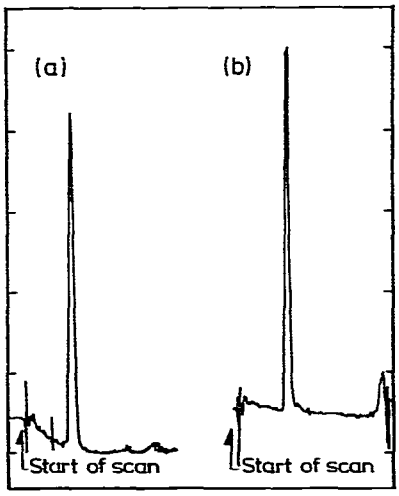
For dimetilan the ratio

$$\frac{\text{area of "spotted" peak}}{\text{area of chromatographed peak}}$$

was 0.97 with a range of 0.76–1.25 and standard deviation 0.14, for bendiocarb the values were 0.85, 0.54–1.10, and 0.15, respectively.

Variability

To determine the variability due to the scanner itself as distinct from that introduced by chromatography, two 1-μl volumes of a solution containing 100 μg



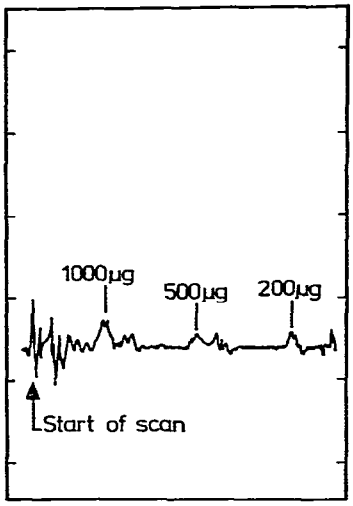


Fig. 2. Scans showing detector response to 100 ng dimetilan (a) developed on rod, and (b) spotted onto rod.

Fig. 3. Scan showing detector response to 200, 500, and 1000 μg formic acid spotted onto rod. Scale is the same as in Fig. 2.

dimetilan were placed at separate positions on the rod, the standing current was adjusted to 1.6×10^{-10} A and the rod was scanned. The areas under the peaks produced by the dimetilan spots were measured. This process was repeated a further 19 times. The mean areas for each of the two peaks were 156 and 168 arbitrary units, with standard deviations of 9 and 12% of the means, respectively. There was therefore a small improvement over the 14% standard deviation observed after chromatographing dimetilan.

Selectivity

To determine the potential and selectivity of the instrument for nitrogen-containing compounds, spots of 1, 0.5 and 0.2 mg formic acid were also placed on a rod and scanned. From the results shown in Fig. 3 it may be seen that 1000 μg formic acid gave less than 4% full scale deflection of the recorder pen. The response to carbamates was greater than that to formic acid by a factor of at least 100,000:1 on a weight basis.

CONCLUSION

The alignment of the rod in the scanner and its position relative to the positions of the jet and rubidium chloride annulus are critical to the performance of the instrument. A small change in geometry causes a large change in response. The poor precision of measurement of dimetilan and bendiocarb may be due in part to the relatively crude construction of the instrument: a considerable improvement in performance could probably be achieved by constructing the instrument to closer tolerances. A second factor may have been the use of volumes of only 1 μl of solution to spot compounds onto the rod. The variability inherent in dispensing such a small

volume could also have contributed to the comparatively poor precision observed.

Further improvements in sensitivity and precision of the instrument could be obtained by the application of a system of programmed multiple development[5] to obtain narrower spots.

The application of a TID to thin-layer chromatograms shows promise for the evaluation of chromatograms of N-containing compounds which cannot be readily estimated by GC. By using other detectors, specificity and sensitivity can be altered.

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