

Short communication

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New detector systems for thin-layer chromatography

Some detectors used in gas chromatography have recently been applied to the detection and monitoring of solute zones on thin-layer chromatograms. The following principles of detection have been presented so far. Thin-layer chromatograms prepared on rods or strips are made to pass through the jet of a flame ionization detector (FID) (ref. 1,2). Alternatively, thin-layer strips are passed gradually through a pyrolytic oven from where the gaseous pyrolysis products are swept by a stream of carrier gas into an FID (ref. 3,4). The latter principle can be applied with great advantage by using a tube the internal wall of which is coated with a thin layer of adsorbent⁵. Such

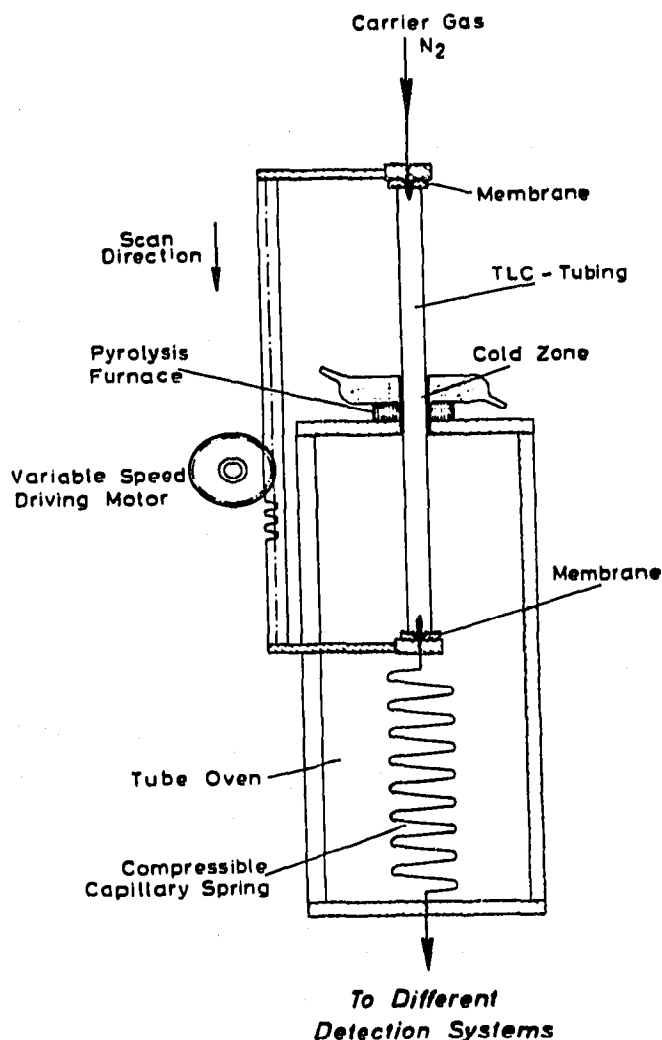


Fig. 1. Driving unit of the thin-layer chromatographic detector. For details, see text.

a tube can also be coated with a mixture of the chromatographic support together with an oxide^{6,7} that effects consecutive combustion of the organic solute zones *in situ* followed by detection of the carbon dioxide formed using a thermal conductivity detector. This method presents an easy means of monitoring thin-layer chromatograms because combustion of solute zones, *e.g.*, in the presence of cupric oxide, requires markedly lower temperatures (600°) compared with pyrolysis (850°). However, pyrolysis offers advantages over combustion owing to undesired effects caused by the oxidant in the chromatographic separation as used in the latter technique.

We wish to report an instrumental system that permits both the pyrolytic⁵ and the combustive^{6,7} monitoring of thin-layer chromatograms. In this system, chromatography is carried out on adsorbent layers that are coated on internal walls of quartz or Pyrex tubings. Subsequently, the pyrolysis products can be detected by the FID, either directly or after their oxidation to carbon dioxide and subsequent reduction to methane. Similarly, carbon dioxide formed in combustive monitoring can be reduced to methane and detected by the FID.

Experimental

Description of the detector. The construction of the vertical driving unit for

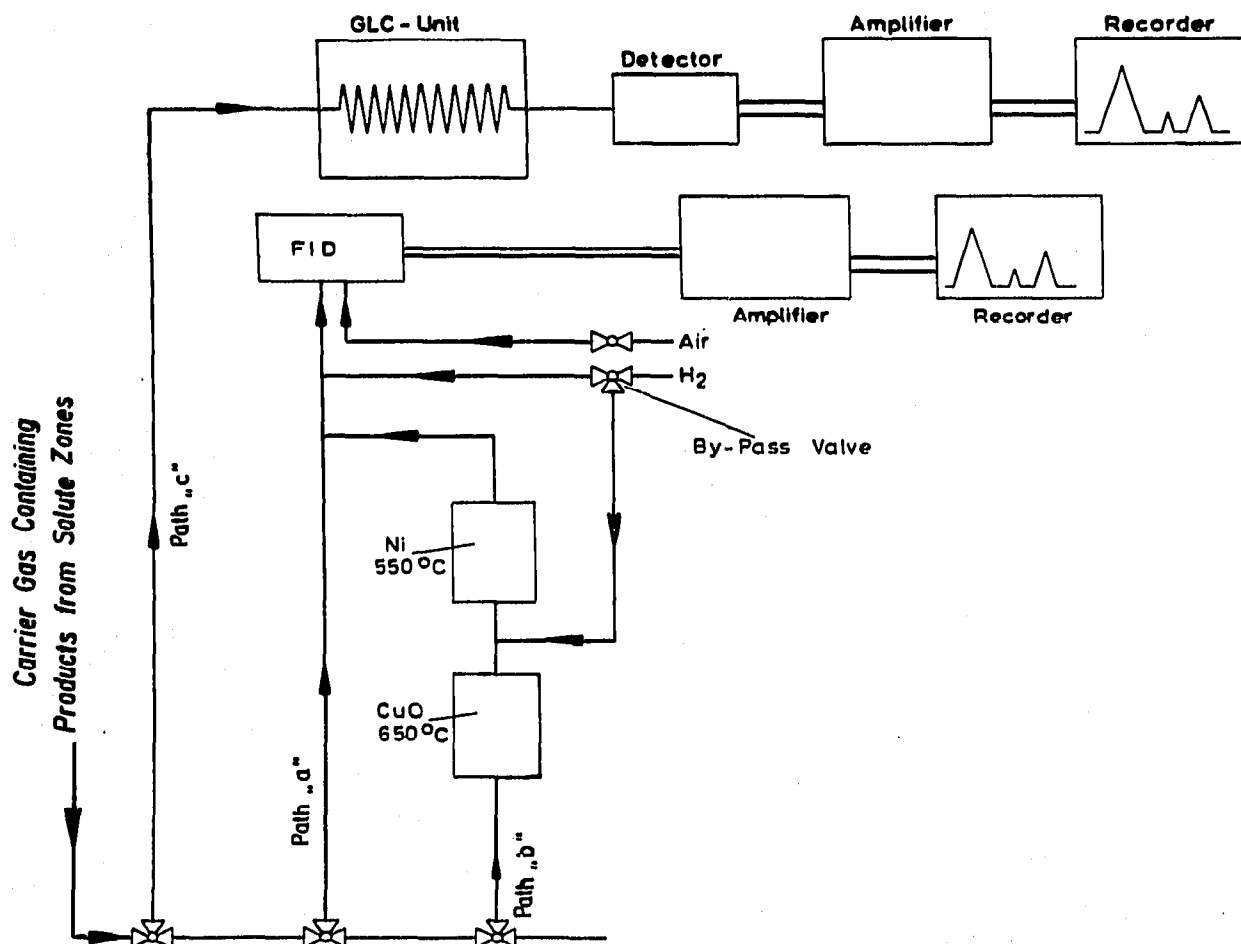


Fig. 2. Flow diagram of the thin-layer chromatographic detector. For details, see text.

scanning the chromatograms is presented schematically in Fig. 1. The upper end of the thin-layer tube, which is internally coated with a thin layer of adsorbent, is connected to the inlet for the carrier gas. A motor drives this tube through a cylindrical pyrolysis or combustion furnace into an oven of adjustable temperature which prevents both condensation of pyrolysis products and their re-adsorption on to the layer. Technically this is achieved through connection of the tubing to a compressible capillary spring which is located inside the tube oven. A cylindrical cooler can be mounted on the pyrolysis furnace in order to achieve a steep temperature gradient. This has been found to be of advantage, especially in pyrolytic scanning. During scanning, solute zones are consecutively vaporized from the thin-layer tube, either by pyrolysis or by combustion, and the resulting products are swept by the carrier gas to various detection systems as shown in Fig. 2.

Operation of the detector. Following path (a) as shown in Fig. 2, nitrogen, the carrier gas, containing pyrolysis products, enters the FID directly through a heated line. Following path (b), the pyrolysis products flow through an "oxidation capsule", e.g.,

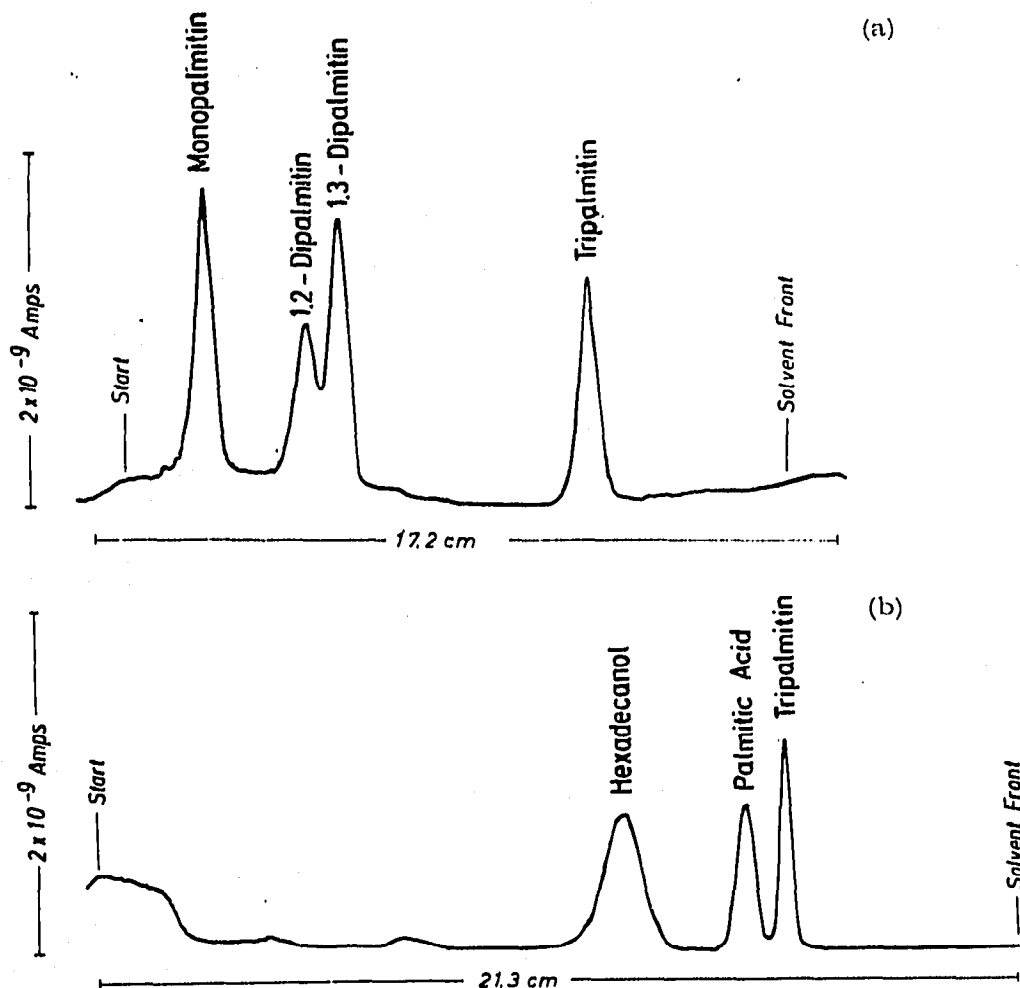


Fig. 3. Pyrolytic scans of thin-layer chromatograms of neutral lipids using (a) path "a", (b) path "b". Layer: Silica Gel G (E. Merck), cleaned up by pre-scanning prior to chromatography. Developing solvent: hexane-diethyl ether-formic acid (85:15:1). Sample size: ca. 15 μ g. Scanning speed: 1 cm/min. Flow rates: N_2 , 45 ml/min; H_2 , 45 ml/min; air, 700 ml/min. Temperatures: pyrolytic furnace, ca. 800°; tube oven and lines, 225°; CuO, 650°; nickel catalyst, 550°.

cupric oxide at 650°, to a "reduction capsule", *e.g.*, nickel catalyst at 550°. Reduction is accomplished by introducing hydrogen at a nitrogen/hydrogen volume ratio of about 1:1. The carbon dioxide formed by combustion is reduced to methane which is detected in the FID. The by-pass valve maintains supply of hydrogen to the detector when the tubing is disconnected. This design prevents the entry of hydrogen into the "oxidation capsule" while the thin-layer tubes are being replaced. Alternatively, path (c) leads the pyrolysis products to a gas-liquid chromatography unit for fingerprinting of the substances formed from a given solute zone.

When combustive monitoring is used, the stream of carrier gas is led through path (b), the carbon dioxide being converted to methane. Combinations for simultaneous detection of $^{14}\text{CO}_2$ and/or $^3\text{H}_2$ are possible in the same manner as reported earlier^{6,7}. If a thermal conductivity detector is employed instead of the FID, as in the earlier detection system^{6,7}, the reduction capsule has merely to be replaced by a water trap.

Results

The instrument has been tested following the above principles of paths (a), (b) and (c). Detailed performance figures have been determined. As a summary of these studies, the following can be stated.

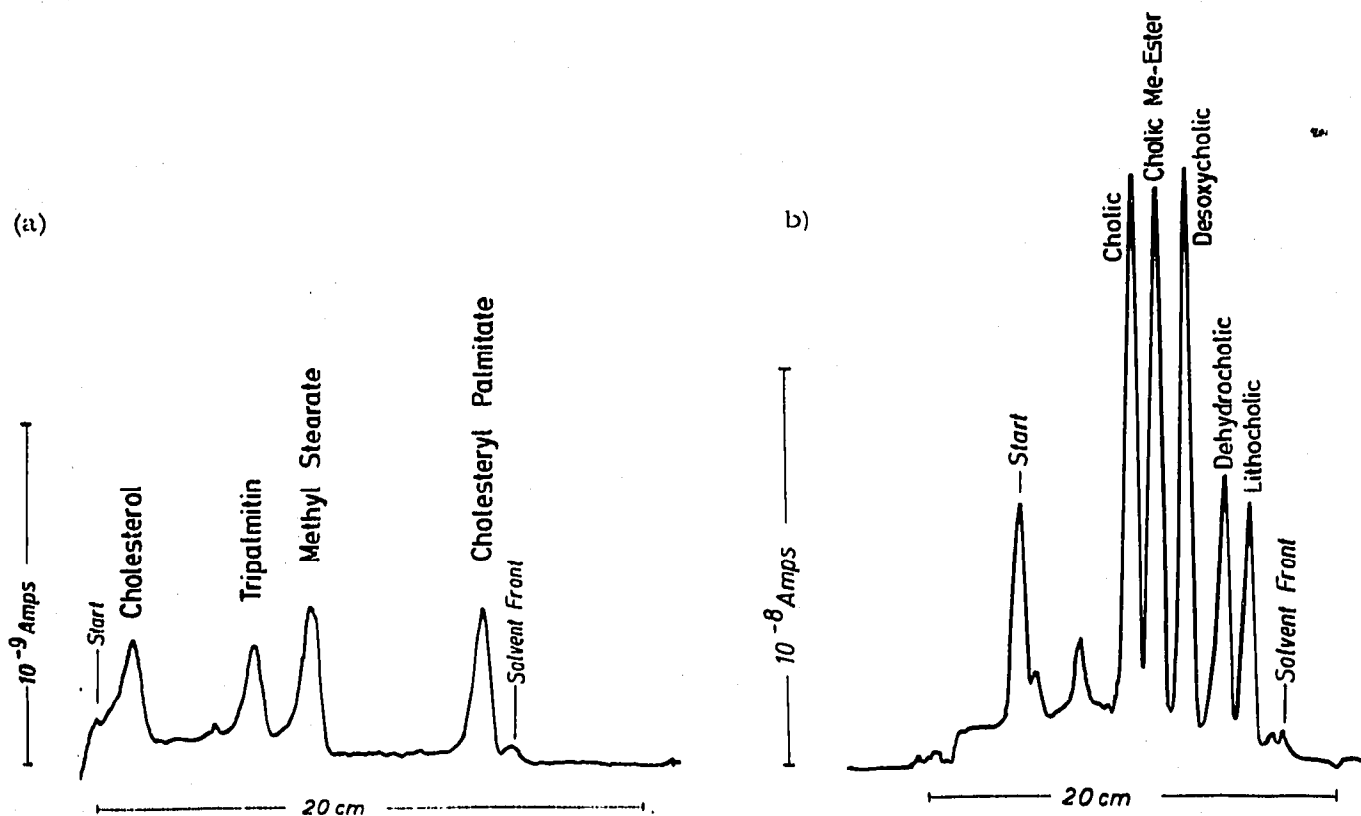


Fig. 4. Combustive scans of thin-layer chromatograms of neutral lipids (a) and bile acids (b) using path "b". Layer: 10% cupric oxide in Supelcosil 12A (Supelco). Developing solvent: (a) hexane-diethyl ether-acetic acid (90:10:1); (b) chloroform-acetone-methanol (90:20:10). Sample size: (a) 5 μg ; (b) 50 μg . Scanning speed: 2 cm/min. Temperatures: combustion furnace, *ca.* 600°; tube oven and hot lines, 150°. All other conditions as in Fig. 3.

The detection system can be used to directly monitor pyrolysis products of a variety of lipids by path (a) or path (b). The use of quartz tubes is obligatory in methods based on pyrolysis. Re-condensation of pyrolysis products on the layer, or their re-adsorption, does not occur at a tube oven temperature of 250–300°. The yield of pyrolysis products is in the region of 95% or even higher. When the method of combustion is employed, Pyrex tubes are adequate.

The oxidation efficiency of cupric oxide at 650°, as tested with methane, is better than 99.9%, and reproducible (S.D. \pm 0.02%). The reduction efficiency of carbon dioxide to methane at the flow-rates tested varies from 70 to 95% at 500°, depending on flow conditions and/or catalyst batch, but is reproducible under a given set of conditions (S.D. \pm 0.1%).

The ultimate sensitivity of the detection system depends upon the FID. Tests we have carried out at an FID efficiency of about 8 mC/g are not at the optimum level. However, the practical sensitivity of the detector is limited by background noise, which is caused by uneven layers and/or organic contaminants. Methods for the preparation of tubes yielding low backgrounds are being devised in order to increase the sensitivity of detection. So far, the quantification of 0.1–0.5 μ g per zone can be considered as the lower limit. Figs. 3 and 4 illustrate typical examples of operation of the detector in pyrolytic and combustive monitoring of thin-layer chromatograms.

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