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Note

A new technique for scanning tubular thin-layer chromatograms

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A novel scanner for quantitative thin-layer chromatography (TLC) has been described earlier[1–4] and has been applied to the analysis of radioactively labelled substances[5] and lipids of amniotic fluid[6]. Mixtures of organic substances are first separated in quartz or glass tubes that are coated internally with a thin layer of silica gel or a mixture of silica gel and copper(II) oxide. Using a Packard Model 7251 TLC Quantuscan scanner (Packard-Becker, Delft, The Netherlands), the substances in the individual zones of the tubular thin-layer chromatogram are vaporized consecutively by either pyrolysis or combustion. This is accomplished by moving the tube gradually through a ring-shaped furnace maintained at 600–800 . An inert carrier gas (nitrogen or helium) flowing through this tube delivers the products of pyrolysis or combustion, either as such or after their conversion into methane, to a flame ionization detector (FID) or a thermal conductivity detector (TCD).

In this device, the quantitative response of the individual components of a mixture depends largely on the extent of vaporization of the substances. In the presence of nitrogen or helium, most organic compounds are completely vaporized from layers of silica gel if sufficiently high temperatures are employed. We found, however, that several groups of compounds, such as certain amphiphilic lipids, steroids and terpenoids, are not pyrolyzed completely even at 800–900 . If copper(II) oxide is incorporated in the layer, all organic compounds are vaporized quantitatively, mainly by combustion. However, as copper(II) oxide is not chemically inert, the tubes that are coated with an adsorbent containing copper(II) oxide cannot be used for certain chromatographic separations, especially if the developing solvent contains a reactive component.

It has now been found that in scanning tubular chromatograms, the use of an oxygen-containing carrier gas such as air offers several advantages over an inert gas. If oxygen is present in the carrier gas, all organic substances studied so far are completely vaporized, mainly by combustion, irrespective of whether silica gel or a mixture of silica gel and copper(II) oxide is used as the adsorbent. Both types of thin-layer tubes can be re-used, in contrast to the earlier systems[3,4] in which the tubes containing copper(II) oxide could be used for a single analysis only.

EXPERIMENTAL

Quartz or glass tubes coated internally with silica gel G (E. Merck, Darmstadt, G.F.R.) or a mixture of silica gel G and copper(II) oxide (10–40 μm; E. Merck) are prepared as described earlier[1,4]. The samples to be analyzed are spotted on the layer with a micro-syringe and the thin-layer tubes are developed by the ascending technique using purified solvents. After development, the solvent is removed at 120–140° in a stream of nitrogen.

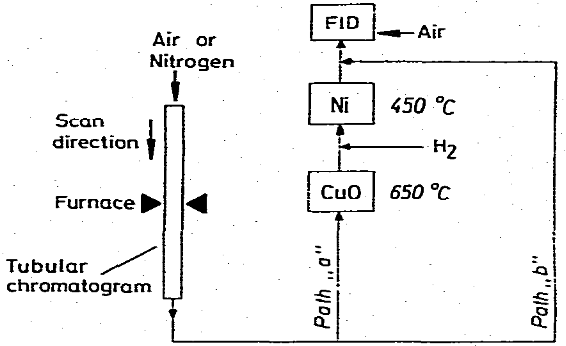

Fig. 1. Flow of air or nitrogen in the TLC scanner.

The tubular thin-layer chromatograms are scanned in a prototype instrument (Packard-Becker, Delft, The Netherlands), as shown schematically in Fig. 1. Purified air or a synthetic mixture of nitrogen and oxygen (80:20) and, for comparison, nitrogen are used as carrier gases. The flow-rates of the carrier gas and hydrogen and air for the FID are adjusted so as to obtain a stable flame. The tubular chromatograms are traversed at a speed of 1–2 cm/min by the stationary furnace, which is maintained at 600–800°. The gaseous products emerging from the tubular chromatogram are passed via path "a" over heated copper(II) oxide, which oxidizes any uncombusted organic material. The carbon dioxide formed is reduced over a nickel catalyst to methane, which is monitored by the FID. Alternatively, the gaseous effluent from the tubular chromatogram is delivered to the detector directly via path "b".

For quantitation, the peaks of the individual chromatographic zones are measured by cutting out and weighing. The relative peak areas are converted into weight-percentages of the substances by taking into account the theoretical yield of carbon dioxide per unit weight of the substance or group of substances concerned.

RESULTS AND DISCUSSION

The extent of vaporization in tubes coated with silica gel has been studied for a large variety of organic substances. It was found that with nitrogen as the carrier gas, certain substances, such as some amphiphilic lipids, terpenoids and steroids, are partly carbonized on the layers, even when rather high temperatures (800–900°) were employed during scanning. Under identical conditions, all of the compounds studied so far were completely vaporized if air or a mixture of oxygen and nitrogen was used as the carrier gas. The resulting silica gel layers were free from carbonaceous material. Such thin-layer tubes can be used repeatedly without any significant alteration in chromatographic properties.

In order to determine whether on silica gel vaporization occurs by pyrolysis or by combustion, a known amount of cholesterol was spotted in several thin-layer tubes that were coated with silica gel. These tubes were subsequently scanned at approximately 800° with either air or nitrogen as the carrier gas. The resulting gaseous products were delivered to the FID directly via path "b" (Fig. 1) and the corresponding peaks were measured. It was found that the peak area with air as carrier gas was only 15–20% of that obtained with nitrogen. As the FID detects neither carbon dioxide nor water, this decrease in response with air as the carrier gas indicates that in the presence of oxygen organic substances are vaporized from the layers of silica gel predominantly by combustion *in situ*, and not by evaporation and pyrolytic fragmentation as in the earlier detection system[3,4].

On layers of silica gel containing copper(II) oxide, all of the organic substances studied so far were completely vaporized, mainly by combustion, irrespective of whether nitrogen or air was used as the carrier gas.

With nitrogen as the carrier gas, the copper(II) oxide in the various chromatographic zones is reduced to metallic copper. Such thin-layer tubes cannot be re-used, for obvious reasons. However, with air as the carrier gas the metallic copper formed by combustion is immediately oxidized back to copper(II) oxide and, therefore, these thin-layer tubes can be used repeatedly. This is a distinct advantage over the system reported earlier[3,4].

In order to determine whether oxygen in the carrier gas affects the conversion of carbon dioxide into methane and the sensitivity of the detector, equal amounts of reference substances such as a sterol or a triacylglycerol were spotted in thin-layer tubes coated with silica gel or a mixture of silica gel and copper(II) oxide. The tubes were subsequently scanned using both nitrogen and air as the carrier gas and the gaseous effluent was delivered to the FID via path "a". The responses obtained in the FID were almost the same irrespective of whether air or nitrogen was used as the carrier gas. This shows that neither the efficiency of conversion into methane nor the sensitivity of the detector is significantly affected by the presence of oxygen in the carrier gas.

Several mixtures of reference compounds were analyzed on layers of silica gel as well as on layers of silica gel containing copper(II) oxide using both nitrogen and air as the carrier gases. The gaseous products from the tubular thin-layer chromatograms were delivered to the FID via path "a". It was found that with air as the carrier gas, the results obtained on silica gel layers were in excellent agreement with those obtained on layers consisting of silica gel containing copper(II) oxide. Furthermore, the tubes coated with mixtures of silica gel and copper(II) oxide yield essentially the same results, irrespective of whether air or nitrogen was used as the carrier gas.

REFERENCES

1 H. P. Kaufmann and K. D. Mukherjee, *Fette, Seifen, Anstrichm.*, 71 (1969) 11.
2 E. Haahti, R. Vihko, I. Jaakonmäki and R. S. Evans, *J. Chromatogr. Sci.*, 8 (1970) 370.
3 K. D. Mukherjee, H. Spaans and E. Haahti, *J. Chromatogr.*, 61 (1971) 317.
4 K. D. Mukherjee, H. Spaans and E. Haahti, *J. Chromatogr. Sci.*, 10 (1972) 193.
5 K. D. Mukherjee and H. K. Mangold, *J. Chromatogr.*, 82 (1973) 121.
6 T. Laatikainen, T. Hokkanen and E. Haahti, *Scand. J. Clin. Lab. Invest.*, 31 (1973) 347.