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## A NEW METHOD FOR THE ANALYSIS OF RADIOACTIVELY LABELLED COMPOUNDS

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### SUMMARY

A new scanner for quantitative thin-layer chromatography can be employed for the measurement of radioactive fractions. Using this instrument, one can assess both the chemical and the radioactive purity of labelled substances. Moreover, it is possible to determine the specific activity of pure labelled compounds.

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### INTRODUCTION

Chromatographic methods are widely accepted for the analysis of labelled compounds. Thin-layer chromatography (TLC) is especially popular owing to its simplicity, speed and versatility<sup>1,2</sup>.

A scanner developed recently<sup>3,4</sup> permits the detection and quantitative analysis of organic solute zones on thin-layer chromatograms. Mixtures of compounds are fractionated in glass or quartz tubes coated internally with a thin layer of adsorbent. Using the TLC-Scanner\*\*, the various chromatographic zones are vaporized consecutively, either by combustion or by pyrolysis, and the gaseous products are monitored with a flame ionization or thermal conductivity detector. Such a device can also be used for monitoring radioactivity continuously if a proportional flow counter is employed<sup>5</sup>. However, it is well known from radio-gas chromatography that such arrangements are not very satisfactory, especially when low activities are to be determined<sup>6</sup>.

Although the TLC-Scanner has been designed basically for monitoring mass, we have found that, in addition, radioactivity in various zones of a chromatogram can be determined accurately as follows. The radioactive products formed by *in situ* combustion of the labelled compounds are absorbed in hyamine solution and the

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radioactivity in each fraction is determined in a liquid scintillation spectrometer. Alternatively, the radioactive products formed by *in situ* pyrolysis are combusted, and the resulting  $^{14}\text{CO}_2$  and  $^3\text{H}_2\text{O}$  are trapped in hyamine and measured.

## EXPERIMENTAL

Chromatography is carried out in a thin-walled tube,  $20 \times 0.5$  cm, coated internally with silica gel or with silica gel containing 15% of copper(II) oxide. The tubular chromatogram is connected to a supply of nitrogen, which serves as the carrier gas. A variable-speed motor drives the chromatogram through a scanning furnace. The products of combustion or pyrolysis are swept by the nitrogen over copper(II) oxide, which completes the combustion of all the organic material. The carrier gas containing  $^{14}\text{CO}_2$  and/or  $^3\text{H}_2\text{O}$  is then bubbled through a series of tubes, each of which contains 1 ml of a 1 M methanolic solution of hyamine hydroxide\*. Using a three-way valve in the path of the gas entering the hyamine solution, the fraction tubes are changed at regular intervals with no significant loss of radioactive effluent. After the entire chromatogram has been scanned, the contents of each tube are transferred into counting vials with 10 ml of scintillation solution (10 g of PPO and 0.6 g of dimethyl-POPOP in 2 l of toluene\*) and the radioactivities of the fractions are measured in a liquid scintillation spectrometer. The device is shown schematically in Fig. 1.

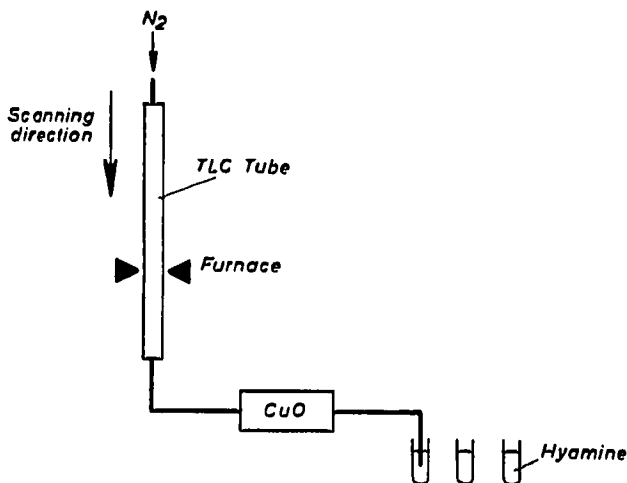


Fig. 1. Scanner for the quantitative TLC of labelled compounds.

In order to monitor the mass of the various solute zones, the products of combustion or pyrolysis are converted into methane and detected with a flame ionization detector, as described earlier<sup>4</sup>.

Fig. 2 shows typical applications of the scanner in the analysis of labelled substances. A sample of  $[1-^{14}\text{C}]$ hexadecylglycerol<sup>7</sup>, specific activity  $7.2 \mu\text{Ci}/\text{mg}$ , was

\* Packard Instrument Co. Inc., Downers Grove, Ill. 60515, U.S.A.

purified by a single crystallization from ethanol. Both the crystalline product and the mother liquor were chromatographed in tubes coated with silica gel containing 15% of copper(II) oxide, using hexane-diethyl ether, 70:30 (v/v), as developing solvent. After removal of the solvent, the tubes were scanned for both radioactivity and mass.

## RESULTS

The results of the analyses of two samples are presented in Fig. 2. The distribution of radioactivity, as determined by liquid scintillation counting of fractions, is

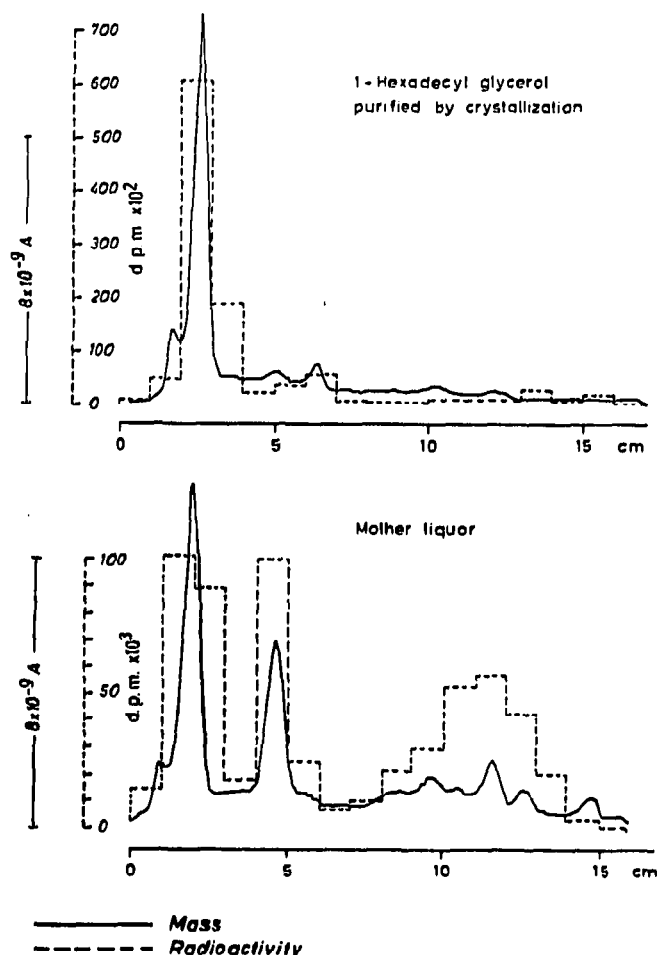


Fig. 2. Distribution of radioactivity and mass in the various zones of tubular thin-layer chromatograms of (a)  $[1-^{14}\text{C}]$ hexadecylglycerol ( $1.1 \times 10^5$  d.p.m.), purified by a single crystallization, and (b) mother liquor ( $6.6 \times 10^5$  d.p.m.) from the above crystallization. Conditions for monitoring radioactivity: scanning speed 1 cm/min; temperature of scanning furnace  $650^\circ$ ; temperature of copper(II) oxide  $650^\circ$ ; flow-rate of nitrogen 40 ml/min. Conditions for monitoring mass: nickel catalyst for reduction of carbon dioxide  $450^\circ$ ; flow-rate of hydrogen 40 ml/min; flow-rate of air 350 ml/min; other conditions as above.

shown by dotted lines. The distribution of mass, as recorded by the flame ionization detector, is depicted by solid lines.

The distributions of both radioactivity and mass indicate that the product obtained by crystallization still contains at least 20% of impurities. In addition to substantial amounts of [1-<sup>14</sup>C]hexadecylglycerol, the mother liquor contains labelled hexadecanol, isopropylidenehexadecylglycerol and dihexadecyl ether.

The crystalline product is purified further by preparative TLC, and both the radioactive purity and chemical purity of the final product are ascertained by the technique described.

## DISCUSSION

The examples given in Fig. 2 demonstrate that both radioactivity and mass in individual chromatographic zones can be conveniently monitored. Simultaneous recording of radioactivity and mass can be accomplished if a stream splitter is used. The absolute determination of mass in a particular zone is carried out by the use of an internal standard or by the injection of a known amount of carbon dioxide in the mass detection system<sup>4</sup>. Thus, both the radiochemical and the chemical purity of labelled compounds can be assessed and their specific activities determined. These are distinct advantages of this technique over methods that involve autoradiography of chromatoplates or liquid scintillation counting of scraped zones.

Under the conditions used, the combustion is found to be complete, and over 95% of <sup>14</sup>CO<sub>2</sub> is absorbed by the hyamine solution. The counting efficiency is reduced by about 30% owing to the presence of hyamine, but the quenching factor remains constant irrespective of the total amount of <sup>14</sup>CO<sub>2</sub> absorbed; chemical quenching is eliminated. This forms a reliable basis for the quantitative measurement of <sup>14</sup>C-labelled compounds. The technique described is applicable also to compounds labelled with tritium.

Further work is being devoted towards mechanization of the trapping and transfer device, which should reduce manual operations to the minimum.

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