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METHOD FOR THE DETECTION OF ORGANIC COMPOUNDS ON PAPER CHROMATOGRAMS WITH HIGH RECOVERY OF THE SAMPLE

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SUMMARY

The proposed method for the detection of organic compounds on paper chromatograms with high recovery of the sample is based on the appearance of dark spots on the chromatogram after heating the paper with, for example, the hot-plate of a domestic iron. The sensitivity of the method could be increased by examination under UV radiation at 366 nm. This extends the detection limits to the nanomole level for many substances. The application of the method gave high recoveries of radioactive glucose and lysine. With glucose it was further found that the chemical properties of the compound were preserved. The procedure is simple and can be carried out in a few seconds without reagents, using inexpensive equipment.

INTRODUCTION

Most of the methods currently used for the detection of compounds on paper after chromatography or electrophoresis involve treatment of the paper with different solutions. The staining in these instances is generally achieved as a result of specific properties of the substances. A thermal procedure was described in 1964 for the identification of some sugars on paper chromatograms[1]. One important aspect of the above methods that still remains unsolved is the quantitative recovery of radioactive substances and the preservation of their chemical properties after detection.

We present here a combination of thermal and UV detection, which extends the detection limits to the nanomole level for many substances. The high recoveries of radioactive glucose and lysine and the preservation of the chemical properties of the glucose after chromatography and the thermal–UV detection are important results of the present work.

EXPERIMENTAL

A domestic iron with automatic temperature regulator was used as the heating source. The temperature was estimated by contact of the hot-plate with the bulb of a standard thermometer.

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Glucose oxidase for enzymatic colorimetric assay of glucose was used as described previously[2].

All chromatographic and electrophoretic procedures were carried out using Whatman No. 1 paper and the following solvent systems: A, *n*-butanol–pyridine–water (6:4:3); B, *n*-butanol–pyridine–water (4:3:4); C, isopropanol–acetic acid–water (27:4:9); D, 95% ethanol–1 *M* ammonium acetate/acid buffer (pH 3.8) (7.5:3); E, 5% formic acid; and F, pyridine–*n*-butanol–acetic acid–water (105:70:21:84). [U-^{14}C]Glucose (250 Ci/mol) and [U-^{14}C]lysine (300 Ci/mol) were purchased from New England Nuclear. Some chromatograms were developed by staining with silver nitrate[3]. The radioactivity in the chromatogram was determined with a Packard Model 7200 radio-scanner.

Spots were observed under UV irradiation near 366 nm. UV photography was carried out using a Fotodyne transilluminator and a normal camera with a Klett dark red filter (650 nm).

Electrophoresis was carried out at 1200 V for 1 h using solvent system E.

Toluene–PPO (2,5-diphenyloxazole) was used for radioactivity measurements in a Beckman Model 8100 liquid scintillation spectrometer.

All standard compounds used were of the best available quality.

RESULTS

The first set of experiments was carried out using 1 μmol of different standard compounds. In all instances descending paper chromatography was performed with the solvent systems for 24 h. The papers were dried in air and developed by ironing

TABLE I

DETECTION OF SOME ORGANIC COMPOUNDS BY THERMAL TREATMENT

Values were obtained from refs. 1, 4 and 5. Conditions described under Experimental.

Substance assayed	*Temperature of darkening or decomposition (°C)*	*Predicted detection*	*Experimentally detected (230°C)*	*Solvent systems*
Cellulose	260–270	–	–	A,B,C,D,E,F
Glucose	146	+	+	A,B,C
Glucosamine	110	+	+	A,B,D,E
Glycerol	290	–	–	C,D
Sucrose	185	+	+	A,B
Lactose	223	+	+	A,B
L-Fucose	145	+	+	A,B,D
Fructose	103	+	+	A,B
Mannose	132	+	+	A,B,C,D,E
Inositol	>319	–	–	A,B,C,D
L-Ascorbic acid	189	+	+	F
Glycine	228	+	+	F
Proline	220	+	+	F
Serine	228	+	+	F
Lysine	210	+	+	F
Succinic acid	233	+	+	D

with the temperature set at 230°C. After 30 sec dark spots were observed. It was possible to continue ironing until maximal contrast was obtained.

Glucose, for instance, which has a decomposition temperature of 146°C, gave a detectable brown spot at 230°C. In contrast, the paper cellulose matrix, with a decomposition temperature of 260°C, did not develop any colour. This difference between cellulose and other compounds with decomposition temperatures below 260°C constitutes the physical basis of the proposed method. It could then be possible to predict the detection of a given substance as shown in Table I. In addition, the

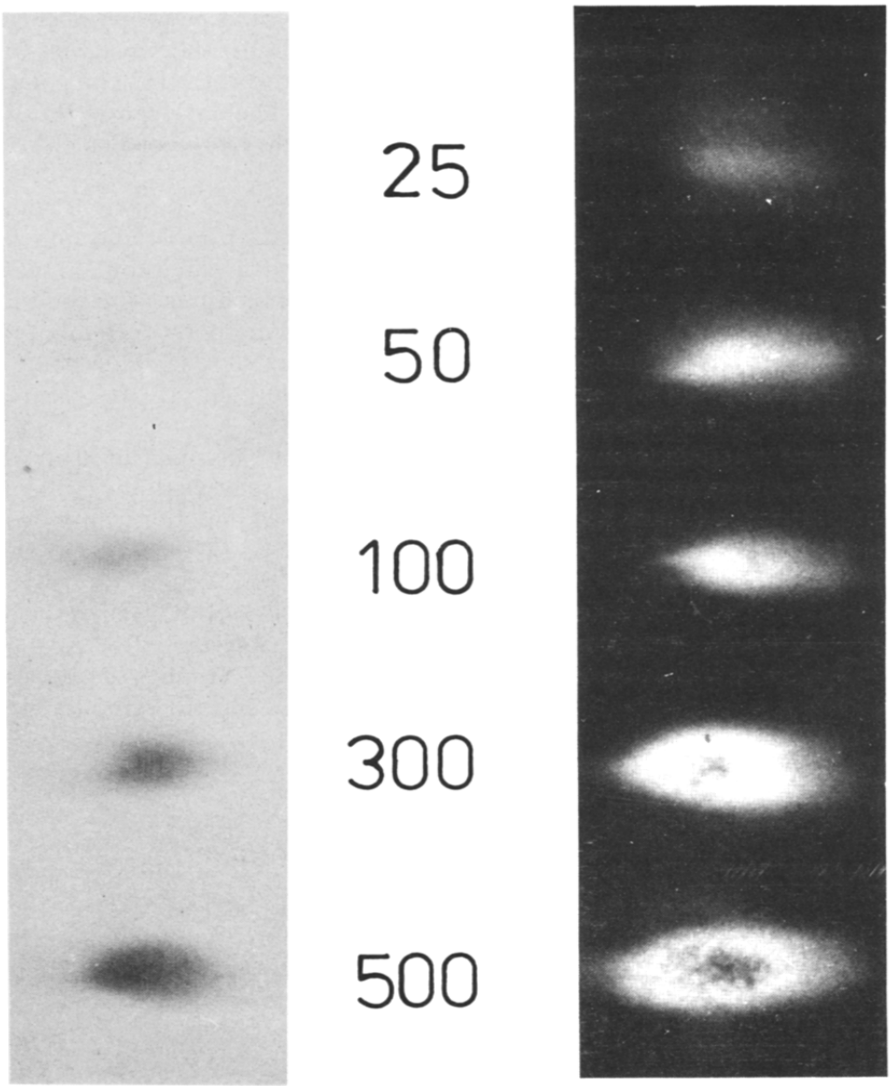

Fig. 1. Photograph of a chromatogram in which different amounts of glucosamine were detected by the thermal–UV method. Left: chromatogram under visible light after heating at 230°C for 30 sec. Numbers indicate nanomoles of glucosamine. Right: same chromatogram observed under UV light (366 nm).

following compounds (not shown in Table I) have been detected: lactose, maltose, malto-oligosaccharides ranging from 2 to 7 units of glucose, galactose, arabinose, ribose, glucose-6-phosphate, glucose-1-phosphate, fructose, N-acetylglucosamine, melibiose, L-rhamnose, xylose, trehalose and citric and malonic acids.

Relatively large amounts of substances were used in the above experiments. In order to investigate the sensitivity of the method, various concentrations of glucose and glucosamine ranging from 25 to 250 nmol were chromatographed separately as described above.

After development of the chromatograms at 230°C for 30 sec, detection of 100 and 200 nmol/cm^2 of glucosamine and glucose, respectively, could be accomplished. The same chromatograms were further observed under UV light; the sensitivies increased to 25 and 50 nmol/cm^2 for glucosamine and glucose, respectively. The glucosamine chromatogram illustrating this test is shown in Fig. 1. The dark centres in the fluorescence spots are possibly due to the quenching of UV light by the brown colour previously developed.

To investigate the effect of the method on the recovery of radioactive compounds the following series of experiments were performed. Equal amounts of [^{14}C]glucose plus 1 μmol of unlabelled glucose were spotted in four lanes on a paper, chromatographed with solvent system A and the following procedures were used to develop the chromatograms (Fig. 2): (a) silver nitrate staining method (dotted bars); (b) the present method at 230°C (black bars) and (c) 150°C for 30 sec (shaded bars). In the last case, detection was accomplished using only UV light. In the control experiment the chromatogram was not developed (white bars). A paper band in a position equivalent to that of glucose was cut. Radioactivity was measured in all instances and the results were expressed as percentage recovery relative to the control. This experiment was repeated using four different amounts of radioactivity as shown in Fig. 2. The mean recoveries of radioactivity were about 98%, 86% and 4% for the thermal method at 150°C for 30 sec (UV), 230°C for 30 sec (visible light) and silver nitrate staining, respectively. In another experiment 12,000 cpm of [^{14}C]lysine plus 1 μmol of unlabelled lysine were spotted on paper and chromatographed in solvent system F. After chromatography the paper was treated at 230°C for 15 sec and the developed spot was marked under UV light. The paper was cut and the radioactivity

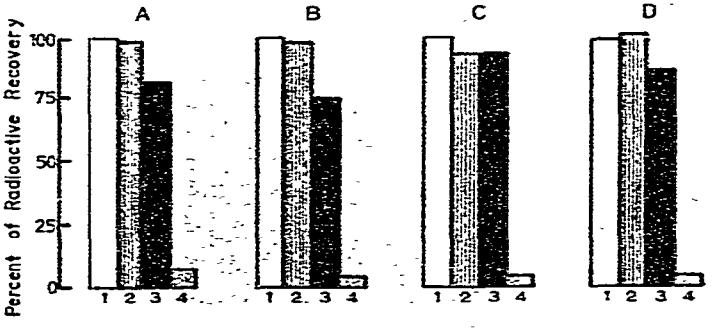

Fig. 2. Radioactive recovery of [^{14}C]glucose after paper chromatography with solvent system A and different detection methods: 1, control (untreated); 2, 150°C, 30 sec, UV; 3, 230°C, 30 sec; 4, silver nitrate staining. Group: A, 8240 cpm; B, 4460 cpm; C, 2860 cpm; and D, 1100 cpm.

measured by liquid scintillation counting. It was found that about 97% of the label could be recovered relative to a chromatographed untreated control.

The chemical decomposition of the radioactive compound after the thermal treatment was studied by examining the chromatographic behaviour of the radioactive substance after thermal–UV detection. A mixture of 1 μmol of unlabeled glucose and 18,000 cpm of [^{14}C]glucose was spotted on paper and chromatographed for 24 h using solvent system A. The paper was treated at 150°C for 30 sec and the sugar was detected using UV light. In an adjacent lane 1 μmol of unlabelled glucose was spotted at the same position as the UV-detected sample, then paper chromatography was carried out for an additional 24 h using the same solvent system. The lane containing the radioactive compound was scanned. The R_F value of the radioactive peak coincided with that of authentic glucose, as shown in Fig. 3. The radioactive spot was cut off and measured by liquid scintillation, and most of the radioactivity (98%) was recovered in the position having an R_F value identical with that of glucose, whereas the remainder of the radioactivity remained at the origin of the chromatogram.

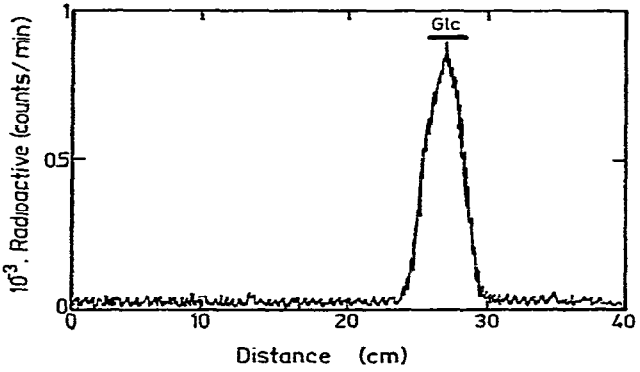

Fig. 3. Scanning of chromatographed [^{14}C]glucose after the sample had been chromatographed for 24 h, detected by the thermal–UV method and rechromatographed for an additional 24 h.

A similar experiment was made using 1 μmol of unlabelled glucose. After chromatography and thermal–UV detection at 150°C for 30 sec, the amount of glucose was measured enzymatically using the glucose oxidase test. It was found that about 96% of the thermally detected compound behaved as authentic glucose.

DISCUSSION

The proposed thermal–UV detection procedure is a simple, sensitive, rapid and inexpensive method for the detection of organic compounds on paper after chromatography and electrophoresis. It allows about 98% recovery of radioactive glucose with preservation of its chromatographic and chemical properties, and about 97% of radioactivity from lysine. The decomposition of a very small amount of sample is sufficient for detection while the rest of the compound is unaffected.

Compared with the silver staining method, the thermal–UV detection had the same order of sensitivity for glucose (50 nmol/cm^2) and was more sensitive for the detection of glucosamine (25 nmol/cm^2). Further, the presence of reducing groups is

not required as in the silver staining method; for instance, glucose-1-phosphate, trehalose, succinic acid and citric acid were detected as easily as glucose.

The chromatographic behaviour of glucose does not appear to be modified after thermal–UV treatment as shown in Fig. 3. Further, up to 96% of the compound obtained after two sequential thermal–UV treatments is still effective as a substrate for the enzyme glucose oxidase (using 1 μmol of glucose). In addition, this compound can be detected by the silver staining method (results not shown).

Our results strongly suggest that different types of substances from those used in this work could also be detected by the thermal–UV method if the following conditions are fulfilled: (1) the compound must decompose or change its colour below 260°C, which is the temperature of decomposition of the paper matrix; this is associated with the appearance of a dark colour; (2) the decomposition should not be accompanied by volatilization. The results obtained with all of the compounds tested in this work appear to conform to these requirements.

Owing to the small amount of decomposition after thermal–UV treatment, the present method could be used in sequential combination with other detection procedures.

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