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Note

Advantage of o-phthalaldehyde for visualising ^{14}C-labelled amino acids on thin-layer chromatograms and an improved method for their recovery

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Ninhydrin has been extensively used for the detection of amino acids on paper and thin-layer chromatograms. The reaction with this compound is, however, accompanied by the loss of the carboxyl group of the amino acid as CO_2 and the formation of an aldehyde which may also be volatile (*e.g.* formaldehyde formed from the reaction of ninhydrin with glycine). When the incorporation of ^{14}C-labelled precursors into amino acids is being studied, it is usually necessary to avoid this loss by visualising the chromatogram by lengthy autoradiographic procedures, or to estimate the proportion of radioactivity lost. Estimation will lead to erroneous corrections when the ninhydrin reaction is incomplete, when the thin-layer plate retains some $^{14}CO_2$, or when the specific radioactivity of the carboxyl carbon atom differs from those of the other carbon atoms in the molecule. The use of a spray reagent whose reaction with the amino acids does not lead to loss of carbon atoms would be preferable. We report that a spray based on the *o*-phthalaldehyde reagent of Benson and Hare[1] has this advantage. Recently a similar spray has been published by Lindeberg[2]. We also describe a simple and convenient method for transferring cellulose scrapings from thin-layer plates to scintillation vials.

EXPERIMENTAL

Square 20-cm thin-layer plates were prepared using a mixture of 30 g Macherey and Nagel MN300 cellulose powder and 190 ml water which had been blended for 1 min. A Baird and Tatlock motorised spreader was used, with a 0.5-mm gap setting. The plates were allowed to dry horizontally at room temperature for about 12 h and then heated at 100° for 30 min.

Aliquots of [U-^{14}C]lysine and [1-^{14}C]glutamic acid were diluted so that 10 μl (a convenient spotting volume) contained about 5000 and 10,000 dpm, respectively. For each spray treatment to be compared, one plate was spotted with three replicate, well-spaced, 10-μl loads of each amino acid over approximately 20 μg of the corresponding unlabelled amino acid as marker, using a cold-air stream to dry. The capil-

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lary micropipette used for spotting was rinsed with three fillings of water after delivery of each radioactive sample, and the washings were spotted around the periphery of each radioactive spot so as not to disturb the cellulose already loaded. All the spotted areas were about 2.2 cm in final diameter.

When the spots were completely dry, one plate was sprayed with a fluorogenic mixture derived by further concentration of the system described by Benson and Hare[1] (omitting Brij). The following components were mixed immediately before use: 25 ml of 0.05 *M* sodium tetraborate; 2 ml of *o*-phthalaldehyde, 10 mg/ml freshly prepared in ethanol; two drops of 2-mercaptoethanol. A light spraying sufficed for maximum development and no heating was required. The spots were visualised by exposure to light of 254 nm. Of the remaining plates, one was left unsprayed. The other was developed with 0.2% (w/v) ninhydrin in ethanol and heated at 100° for 5 min. Finally all plates were separately heated at 100° for 1 h.

The amino acid zones were transferred to scintillation vials containing 5 ml of non-aqueous scintillation fluid, comprising 8 g butyl-PBD/1 toluene. A modification of the recovery method originally described by Blackwood[3] was used (see Fig. 1). The filter paper disc was sucked against the filter holder using a vacuum pump. The cellulose zone to be recovered was scraped from the glass surface of the plate with a spatula and the particles sucked against the filter paper disc. The scrapings were then transferred with the assembly to the mouth of the scintillation vial and deposited into the scintillation fluid as shown in Fig. 2. The successful operation of this system requires that the filter holder fits the mouth of the scintillation vial easily but not loosely. Note that there is no risk of radioactive cellulose being drawn into the vacuum system because the filter paper disc closes across the mouth of the vial. After withdrawal of the filter holder, a plastic cap was inserted into the vial and this pushed the filter paper disc into the scintillation fluid.

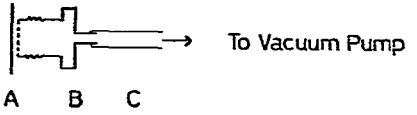

Fig. 1. Vacuum recovery device. A = Whatman No. 1, 2.1-cm filter paper disc; B = the "male" half of a 1.3-cm diameter Sartorius Syringe Filter Holder (V. A. Howe, London, Great Britain); C = flexible plastic tubing.

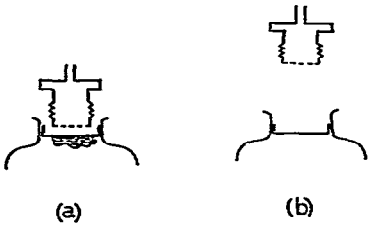

Fig. 2. Release of cellulose scrapings into scintillation vial. (a) Vacuum still on, filter holder pushed into mouth of vial. (b) Vacuum turned off and filter holder removed; cellulose scrapings drop into scintillation fluid.

The radioactivities of 10-μl aliquots of the amino acid solutions were also determined by direct counting in aqueous scintillation fluid (8 g butyl-PBD in a mixture of Triton X-114–xylene (1:3, v/v). All samples were counted in a Beckman LS-250 Liquid Scintillation System, for 20 min each (statistical 2-sigma counting error 0.5% or less). Counting efficiencies were 90–95% and therefore no corrections were applied for quenching.

RESULTS AND DISCUSSION

The mean radioactivities of the lysine and glutamate spots after the different spraying treatments are shown in Table I, together with the mean deviations. The radioactivities recovered from the unsprayed plate were the same as the values determined by direct counting in aqueous scintillant. This shows that the material was recovered from the plate with an efficiency of 100%. The same radioactivity values were obtained from the plate sprayed with the fluorogenic reagent, whereas substantial losses resulted from spraying with ninhydrin. In contrast to ninhydrin, therefore, the *o*-phthalaldehyde spray does not cause a loss of radioactivity from amino acids labelled with ^{14}C. The sensitivity of the spray is probably similar to that reported by Lindeberg[2] since we obtained the same detectable limit for 1 μl of threonine solution spotted on cellulose (100 pmole). We have routinely used this fluorogenic spray to visualise the amino acids from plant extracts separated on thin layers of cellulose with reproducible subsequent recovery of radioactivity.

TABLE I

RADIOACTIVITIES RECOVERED FROM DEVELOPED THIN-LAYER PLATES

Spray Treatment	*Radioactivity of [U-^{14}C]lysine spot (cpm)* *	*Lysine recovery (%)* **	*Radioactivity of [1-^{14}C]glutamate spot (cpm)* *	*Glutamate recovery (%)* **
Not sprayed	6238 ± 3%	96	11 475 ± 4%	106
Fluorogenic spray	6160 ± 1%	95	11 914 ± 2%	111
Ninhydrin spray	3998 ± 6%	62	4 702 ± 2%	44

* The mean of the radioactivities from three spots, corrected for background radioactivity.

** Radioactivity recovered from the plate relative to radioactivity determined directly in aqueous scintillation fluid.

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