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Short communication

Simple, inexpensive system for using thin-layer chromatography for micro-preparative purposes

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Abstract

Glass high-resolution TLC plates were used to separate very small amounts of material (ng–mg). Either spots or linear zones can be quantitatively eluted after separating them from the surroundings with a fast-moving drill, which removes thin lines of layer material. Elution is carried out by siphoning eluent through a specially formed sintered glass on to one end of the zone or spot and picking up the eluate at the other with a piece of filter-paper carton. The latter can be extracted by soaking and centrifugation. Application in the carbohydrate field is demonstrated by a preparative isolation for structural analysis of three components and the determination of different amounts of one compound for analytical purposes.

1. Introduction

Separation, purification and isolation of chemical products from complex mixtures in minute amounts has been the aim of generations of chemists, especially those working with natural products and biochemists searching for metabolites. Of all micro-scale methods, TLC is by far the most popular owing to its simplicity, flexibility in trying out conditions and speed. This is especially so since industry has developed coatings, mostly silica gel, which are equal to GLC in their resolving capacity. Working with small amounts (ng–mg) of complex mixtures of radioactively labelled compounds and compounds carrying chromophor or fluorescent groups, we apply TLC or high-performance (HP) TLC plates, depending on the amount and separability of the mixture to be resolved. A procedure generally used is to scrape off the coating with the adsorbed compound and extract this compound [1].

2. Experimental and results

2.1. Chromatography

Commercial glass TLC plates (Merck, Darmstadt, Germany) are used for the separation of a mixture of compounds. The substances should normally be visible, or made visible under UV radiation or by autoradiography. There is also the possibility of detecting the compounds with a

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reagent on part of the plate and to mark the corresponding zones for elution. Here the best approach is to cut a narrow strip off the plate for detection. In this paper we describe a preparative application with an artificial mixture of three monosaccharides, D-glucose (Glc), D-galactose (Gal) and D-N-acetylglucosamine (GlcNAc) and an analytical application by establishing a calibration graph for a maltose derivative to follow the rate of enzymic maltose liberation.

For the purpose of demonstrating the stages of the procedure in Figs. 1 and 2 a mixture of three dyes was applied to a TLC plate, clearly visible in daylight.

2.2. Zone extraction

The zone to be extracted has to be separated from its neighbours by cutting lines into the coating with a microdrill and then totally remove the adsorbent from the glass support (Fig. 1). A vacuum pipe should be used to suck up loose silica gel dust, especially when the compounds separated are toxic or radioactive.

In a case, which can be tightly closed with a glass plate (Fig. 2) to ensure saturation with the solvent, the TLC plate is placed on a rectangular plateau flanked on the sides with two basins containing the solvent. With a specially cut

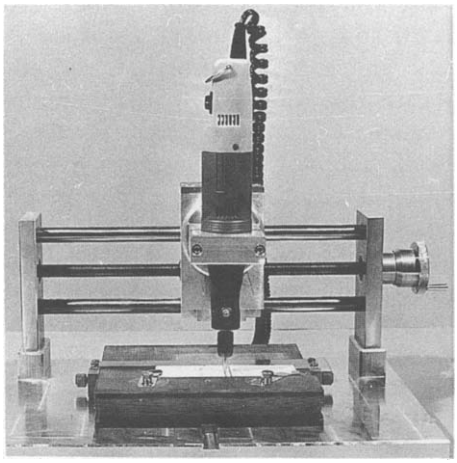

Fig. 1. Microdrill for the separation of zones.

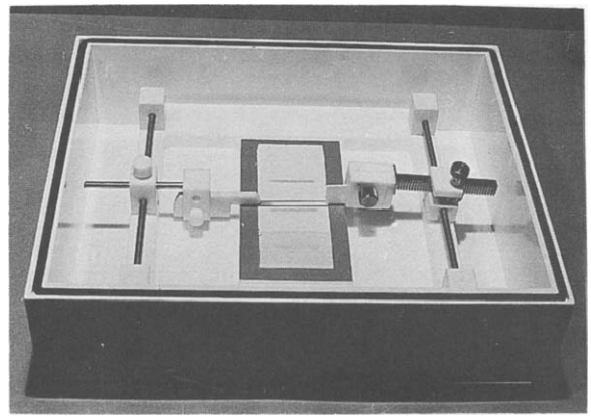

Fig. 2. Elutor for the elution of chemical substances from commercially available TLC and HPTLC plates.

sintered glass the solvent is applied to one end of the zone, where elution of the compound begins. At the other end a rectangular piece of filter-paper carton (Whatman 17 Chr, 3×1 cm) is positioned with one tip touching the coating. The extract is collected in the paper in a circular zone. Extraction is usually quantitative when the radius of the wet segment is ca. 0.5 cm.

2.3. Filter-paper extraction

The paper segment containing the compound is cut into strips (ca. 1 mm) and placed upright in a PTFE cartouche, which has a small perforation in the lower tip and fits into an Eppendorf reaction vessel (Eppendorf–Netheler–Hinz, Hamburg, Germany). The paper strips are soaked with an appropriate solvent mixture (a mixture of water and methanol or ethanol dissolves a great number of different products, especially carbohydrate derivatives). Usually 0.1 ml is optimal. After 10 min the solution is centrifuged for 3 min in an Eppendorf Model 5415 C desk centrifuge into the reaction vessel. This procedure is repeated twice, to ensure quantitative extraction of the compound. Centrifugal freeze-drying (SpeedVac concentrator; Savant Instruments, Farmingdale, NY, USA) yields a uniform compound, without any measurable loss, as could be demonstrated with radioactively labelled substances.

Including concentration to dryness, which con-

sumes the most time, the procedure, starting with the chromatographic separation, lasts not longer than 3 h.

2.4. Application

Preparative

Three monosaccharides (Glc, Gal, GlcNAc), each ca. 2 mg (together not more than 8 mg), representing a typical mixture of a poly- or oligosaccharide hydrolysate, are derivatized with 4-nitroaniline (4-NA) in dimethyl sulphoxide (DMSO) and aqueous formic acid as catalyst by mixing three solutions, a, b and c [a = 1 mg of reducing sugar–40 μl of DMSO; b = formic acid–water (9:1); c = 69 mg of 4-NA–100 μl of DMSO] in the ratio 4:1:2 and heating at 95°C for 75 min as described previously [2]. The product mixture, still containing relatively large amounts 4-NA, is applied to TLC plates (10 × 10 cm, 0.25- or 0.5-mm layer). The solution should be not more than 5% (w/v) and should be applied in only one stroke.

Separation is carried out using ethyl acetate–methanol–water (7:2:1). Three zones (I, II and III) are visible under UV–radiation (254 and 320 nm). The zones [I = 4-NA, R_F 0.75; II = N-4-nitrophenyl-β-D-glucopyranosylamine, R_F 0.57; III = N-4-nitrophenyl-β-D-galactopyranosyl- and N-acetyl-β-D-glucopyranosylamine, R_F 0.53 (these two cannot be separated under these conditions)] are separately eluted [methanol–water (3:1)] and isolated as described in Sections 2.2 and 2.3.

The sugar derivatives from zones II and III are converted into their per-O-acetates in the conventional way [3], using 100 μl of acetic anhydride–pyridine (1:1, v/v). Excess reagent is decomposed with a few drops of methanol and the reaction mixture is evaporated by centrifugal freeze-drying (see Section 2.3).

N-4-Nitrophenyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylamine (**1**) the compound from zone II is uniform as such and its structure can be determined by ^{1}H NMR spectroscopy. The acetylated compounds from zone III according to TLC in ethyl acetate–cyclohexane (1:1) separate N-4-nitrophenyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranosylamine (**2**), R_F 0.3, and *N*-4-nitrophenyl-3,4,6-tri-O-acetyl-2-N-acetyl-β-D-glucopyranosylamine (**3**), R_F 0.13, which can be separately eluted.

To elute the hydrophobic acetates from silica gel, ethanol–chloroform (1:1, v/v) is used. The overall yields of the acetates, which are uniform by HPTLC and pure by ^{1}H NMR of a standard, starting from the mixture of free monosaccharides, are **1** = 84%, **2** = 80% and **3** = 73%. Considering the two chemical reactions, these yields are acceptable. The extraction procedures are essentially quantitative, as could be demonstrated with a radioactive sample of [^{14}C]-D-glucose derivative.

Analytical

Six samples of aqueous solutions containing from 2.69 to 0.084 mg of maltose [4] were evaporated by centrifugal freeze-drying. To each sample the reagents DMSO (40 μl), solution b (10 μl) and solution c (20 μl) were added and treated as described in Section 2.4. After diluting the reaction mixtures each with the same amount of methanol (140 μl), aliquots (1 μl) were applied as spots on the concentration zone of HPTLC plates, separated by ca. 2 cm and chromatographed using ethyl acetate–methanol–water (7:2:1). The product N-4-nitrophenylmaltosylamine (R_F 0.39) is clearly visible under UV radiation. The zones containing the derivative are eluted and worked up as described in Sections 2.2 and 2.3. The isolation procedure is quantitative [the yield of isolated compound is 89% (calculated on the basis of the initial amount of maltose and measured by absorption at 375 nm)]. Measuring the UV absorption at 375 nm and plotting it against the initial amount of maltose gives the calibration graph shown in Fig. 3. If the sugar concentrations are significantly lower (ca. ten times lower than the lowest value in Fig. 3), the optical properties of the N-4-nitrophenylglycosylamines are not sensitive enough for reliable determination. In such a case the formation of dansylhydrazones [1] is advisable.

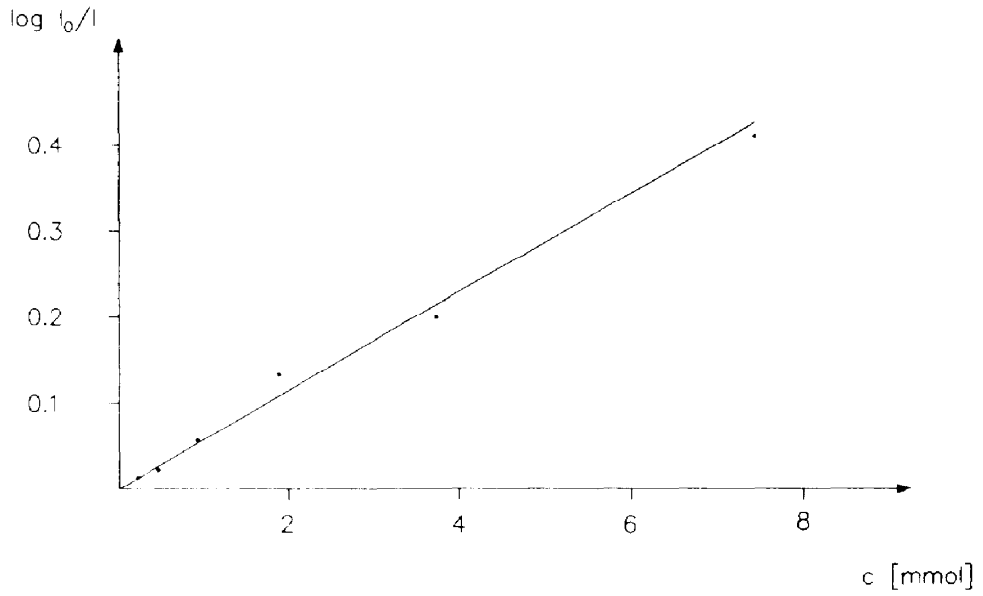

Fig. 3. Calibration graph for N-4-nitrophenylmaltosylamine, measured at 375 nm.

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