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Apparatus for reversed-phase chromatography on small-scale thin-layer plates

Chromatography on microscope slides has become a well established technique in analytical chemistry[1-5]. In our laboratory, special attention has been paid to the reversed-phase application of this technique. However, over the years, the procedure reported in our early papers[2,3] has been improved and changed in many respects. In the present communication, the current procedure and apparatus required are described. As an illustration of the efficiency, we can add that a single analyst can easily prepare and handle 100 chromatoplates, each containing 2–6 sample spots, per day.

Suspension

The suspension is prepared by thoroughly mixing silica gel (Type DO, Fluka, Buchs SG, Switzerland, or Kieselgel DC, Woelm, Eschwege, G.F.R.) or microcrystalline cellulose (Avicel, Macherey, Nagel and Co., Düren, G.F.R.) with a solution of the exchanger in chloroform; 0.17 g (Fluka) or 0.25 g (Woelm) of silica gel, or 0.25 g of cellulose, are used per ml of chloroform solution.

The exchangers used in our studies include liquid anion exchangers (high-molecular-weight amines and substituted quaternary ammonium salts[2,3]), tertiary amine and arsine oxides (Alamine 336-S oxide and tri-*n*-octylarsine oxide[6,7]) and neutral organophosphorus compounds (tri-n-octylphosphine oxide and tri-*n*-butyl phosphate[7,8]). The molarity of the solution is generally *ca.* 0.1. The exchangers are converted into the appropriate salt form by shaking their solutions in chloroform with an aqueous solution containing an excess of the acid used as eluant. With the organophosphorus compounds, however, this equilibration step is often omitted[7].

The suspension is stored overnight and agitated again before use; this improves the quality of the thin layers. Suspensions are discarded when they are one week old.

Thin-layer plates

Chromatoplates are conveniently prepared by dipping ordinary microscope slides (7.6 × 2.6 cm) into the suspension. Dipping by hand yields thin layers of good quality. When a large series of chromatoplates has to be prepared, use of the device shown in Fig. 1a offers a more efficient procedure. Two microscope slides held together firmly with a clip, are pushed down (see arrow) until they are submerged in the suspension for about two-thirds of their height. On release, the *ca.* 100-g weight causes a regular withdrawal of the slides from the suspension.

The pair of coated slides is detached from the clip and placed on a rack, on a piece of filter-paper (Figs. 1b and 2a). On leaving the slides for some minutes in the air, in order to evaporate off the chloroform, a film of impregnated silica gel or cellulose powder adheres to the slides. After a series of chromatoplates has been prepared, superfluous material is wiped off the back of the slides with a piece of plastic foam mounted on a cam (Fig. 1c and Fig. 2b, arrow 1); the waste material is collected in the trough. Subsequently, a series of scores is made in the thin layer by moving the chromatoplate over the row of seven equidistant needles that protrude sideways from

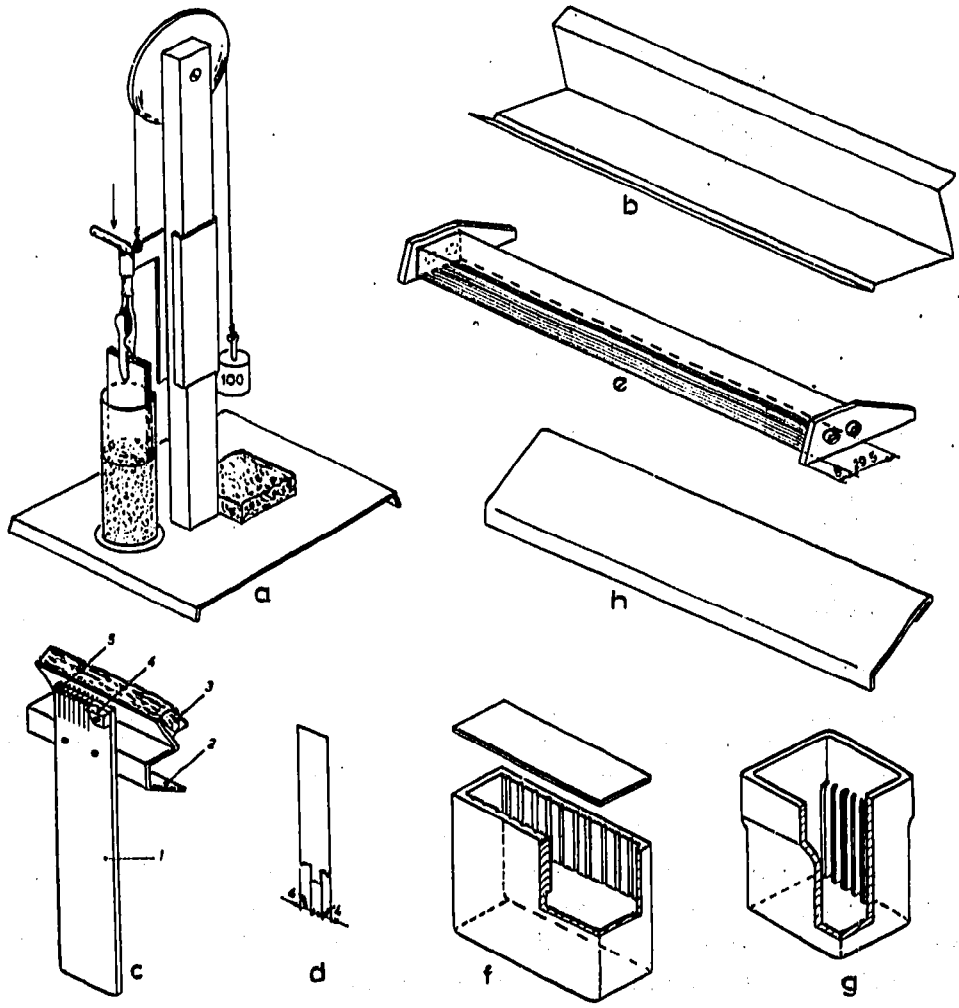

Fig. 1. Apparatus for reversed-phase chromatography. (a) Dipping apparatus. (b) Rack, stainless steel. A rack of *ca.* 30 cm length accommodates 10 chromatoplates. (c) Cam with hard PVC beam (1); aluminium trough (2); piece of plastic foam (3); adjustable stainless-steel block (4); and row of seven equidistant stainless-steel needles (5). The needles are separated by long slots milled in the PVC beam. (d) Device for applying margins along the edge of the chromatoplate; stainless steel. (e) Ruler with graduated beam; Perspex. (f) Elution vessel with lid; Perspex. A vessel of 9 × 8 × 3 cm can hold 10 chromatoplates. (g) Hellendahl staining jar; glass, six plates per jar. (h) Cover (Perspex) lined on the inside with filter-paper.

the top of the cam (Fig. 1c and Fig. 2b, arrow 2). The block next to the needles serves as a guide. As a result, six *ca.* 3.5-mm wide tracks appear on the chromatoplate, and small margins are left along the edges (*cf.* Fig. 2e). The chromatoplates are placed on the rack as shown in Fig. 2d.

For some work, we prefer to apply only 2–3 spots per chromatoplate. In such a case, the drawing of scores is omitted and margins are made using the device shown in Fig. 1d (see also Fig. 2c and section on *Elution*).

Spotting

A ruler (Fig. 1e) is now placed over the chromatoplates; its beam comes close to the surface of the thin layer, but does *not* touch it. A pointed wick of stiff filter-paper, impregnated with the sample solution, serves to apply the spots (diameter

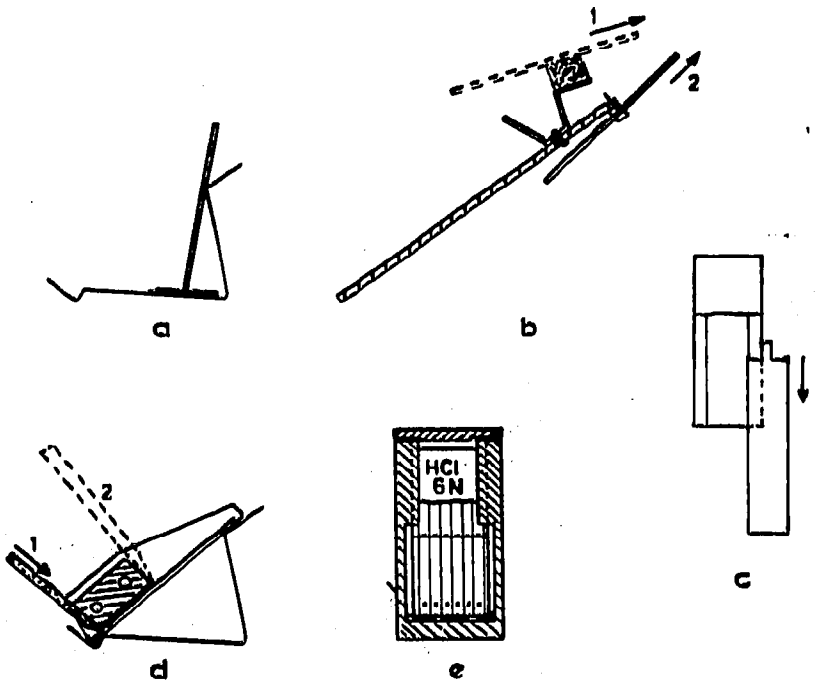

Fig. 2. Various stages of the procedure. (a) Position of the chromatoplate during evaporation of chloroform (used in pairs), and when drying after elution. (b) Wiping off the back of the slide (1) and drawing of scores (2). (c) Application of margin. (d) Spotting procedure (1) and drawing of front groove (2). (e) Elution.

ca. 1 mm; Fig. 2d, action 1). The distance between the spots and the bottom edge of the chromatoplate is 8 mm (*cf.* Fig. 1e). Next, a front groove is made in the thin layer by moving a sharp-pointed pencil or a needle along the top edge of the beam (Fig. 2d, action 2). The width of the beam (29.5 mm) ensures a length of run of 30 mm*.

Elution

Ascending chromatography is performed in Perspex vessels (Fig. 1f), which are filled with eluant to a depth of *ca.* 3 mm. V-shaped grooves, milled in the thick upper part of the long sides, provide a convenient means of supporting the chromatoplates. Their relative distance of *ca.* 7 mm prevents excessive capillary rise of the liquid between the plates. Fig. 2e shows the situation at the start of the elution.

If commercially produced tanks are preferred as elution vessels, the use of Hellendahl staining jars (Fig. 1g) is recommended. However, the long sides of these jars are equally thick over their entire height. Therefore, fairly wide (4 mm) margins must now be made along the edges of the chromatoplates in order to prevent damage to the plates owing to contact with the wet walls of the Hellendahl jar. The alternative procedure described with Figs. 1d and 2c may be used for this purpose. Distortion of the chromatograms owing to uneven flow of the eluant sometimes occurs when this method is used.

The elution is terminated when the eluant reaches the front groove. The chromatoplates are removed from the tank, placed on the rack in the manner shown in Fig. 2a

* Inevitable deviations of the position of the pointed wick and the pencil from the ideal one (touching the face of the beam) cause an increase of the length of run of *ca.* 0.5 mm.

and allowed to drain. In order to prevent possible corrosion, a piece of plastic foil is now inserted between the filter-paper and the rack. If the plates are not air-dry after *ca.* 15 min, the eluant is driven off with a current of hot air. In this way, undue diffusion of the spots is prevented.

Identification; R_F

For identification and R_F measurements, the position of the chromatoplates is changed to that shown in Fig. 2d. Identification is usually achieved by spraying; since this is a well established technique, it will not be discussed here. If an additional treatment with, *e.g.*, ammonia or acetic acid vapour is required, a cover (Fig. 1h) lined with filter-paper impregnated with the appropriate reagent is placed over the slides. Alternatively, the rack with the chromatoplates is placed in a large tank filled with the required vapour.

The ruler in Fig. 1e now serves as the R_F meter. For this purpose, nine equidistant lines (3.0 mm apart) were engraved on the bottom of its beam, thereby graduating it in tenths of an R_F-unit*; the $R_F = 0.5$ line was made somewhat heavier. The ruler is placed over the chromatoplates in the manner shown in Fig. 2d and the R_F values are read off. In order to ensure good visibility of the spots, it is recommended that a piece of thin white paper be inserted between the chromatoplates and the rack.

Cleaning

The chromatoplates are cleaned while they are still standing on the rack, by rinsing them with running tap-water. Subsequently, the rack is immersed in crude acetone, and the chromatoplates are brushed with a reagent-tube brush. After a second dip in pure acetone and subsequent drying in the hood, the rack and slides are ready for the next series of experiments.

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* As the width of the beam is 29.5 mm instead of 30.0 mm, the ranges $R_F = 0.0$–0.1 and $R_F = 0.9$–1.0 are 2.75 mm instead of 3.0 mm. This does not interfere with the measurements.