washing. This meant that the polyamide layer did not change its sorption characteristics after several developing and washing processes.

The wash solution (A) was good for acidic substances. The solution (B) was excellent for basic and neutral types of samples. The dipping time in wash solution (A) should not exceed more than 6 h, otherwise the polyamide layers would peel off. In wash solution (B), the sheets were not harmed even when left for over 3 days.

It was found that the layers should be dipped into the wash solution immediately after development and location of the spots. The samples will be sorbed irreversibly after long standing (for example, overnight).

It is clear from these results that polyamide layers could be used repeatedly. In theory, they can be used an infinite number of times, but mechanical damage caused by handling and decomposition of polyamide resin by developing solvents will restrict it to somewhere around ten times. We have used washed layers in quantitative analysis of DNP-amino acids successfully[8].

This work was partly supported by a Chemistry Research Center grant (No. CRC 5708) to which thanks are due.

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Received July 2nd, 1968

J. Chromatog., 37 (1968) 353–354

CHROM. 3703

Simple preparative thin-layer chromatography

Numerous attempts have been made to exploit the advantages of thin-layer chromatography for preparative work. Such methods are inconvenient, quite apart from the increase in scale required. Thus to elute the components of mixtures directly from chromatograms needs special apparatus and procedures[1,2] and to recover components by extraction involves subsequent removal of fine adsorbent particles. For best results prior concentration "on the layer" by additional chromatographic steps[3,4] is needed. Consequently most workers still use thin-layer chromatography only for analysis and for preparative work move to classical column chromatography[5]

or to the newer "dry column" method[6]. In the two methods described here these difficulties are avoided, thus opening the way to more widespread use of thin-layer chromatography as a preparative tool. Method No. 1 is extremely simple and method No. 2 though slightly more complex has the advantage of being able to cope with about ten times the load per run.

Method No. 1 needs only a chromatography tank with an upper solvent reservoir (as used for descending paper or thin-layer chromatography) and pre-coated thin-layer sheets with flexible plastic backings (alumina, cellulose and silica adsorbent layers may all be used) such as the "Polygram" and "Chromagram" ranges produced by Macherey Nagel and Eastman, respectively. The chromatogram sheet is prepared for use as follows. Edge effects during development are minimised by scraping off 0.5 cm strips of adsorbent from the vertical edges of the sheet (the completed chromatogram sheet is shown in Fig. 1). To ensure that solvent drops will readily fall from the sheet during development its bottom edge is trimmed slightly obliquely leaving at the lowest corner a very sharply pointed 1.0 cm long "tail". Resolution of closely spaced components is aided if adsorbent is scraped off to form a straight lower adsorbent edge. A right angled fold is now made parallel to and 3 cm from the upper edge of the sheet. The adsorbent layer should be on the inside of the fold to minimise peeling of the adsorbent. A further right angled fold is made parallel to and some 1.5 cm down the sheet from the initial fold. These two folds constitute a "hook" by which the sheet may be hung over the lip of the solvent trough. The developing solvent is chosen with the aid of test strips and direct transfer to the preparative system is possible. Very volatile solvents should be avoided to minimise the risk of the chromatogram drying out. Resolution is often improved by multisolvent development with the mobile components being eluted first by non-polar solvents. The chromatogram is run as follows. The sample is applied as a streak terminating some 1.0–2.0 cm from the edges of the sheet to limit edge effects. The loaded sheet is hooked over the edge of the empty solvent trough and a wire clip placed over the "hook" to prevent it opening

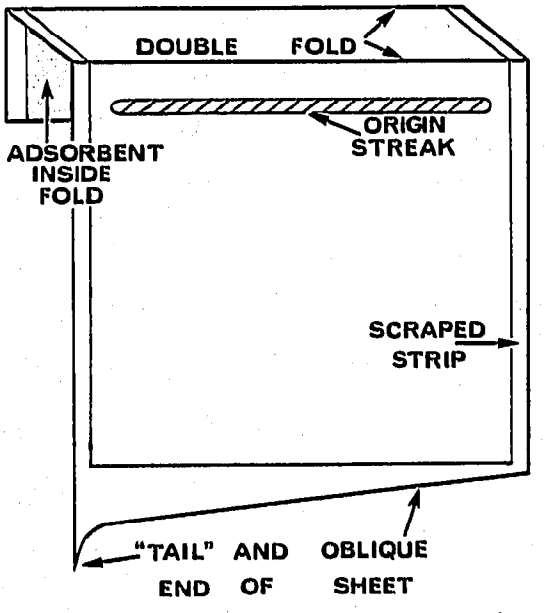

Fig. 1. Completed chromatogram sheet for simple preparative thin layer chromatography using method No. 1.

out during development. Development is started by filling the solvent trough through a previously stoppered port in the tank lid. Separate fractions are collected by moving the sheet along the trough in such a manner that the "tail" delivers successive fractions into successive collecting tubes standing on the floor of the tank. The development of colourless fractions may be followed by using adsorbents containing fluorescent indicators and an ultra-violet lamp arranged to shine through a port in the tank lid. In this case ultra-violet adsorbing aromatic solvents such as toluene cannot be used. A paper wick on the inside of the "hook" will increase the development rate though care must be taken that excessive siphoning and thus irregular flow does not occur.

Method No. 2 at the price of a slight increase in complexity circumvents the major disadvantages of method No. 1, that since the adsorbent layers on pre-coated sheets are only some 0.01 cm thick the useful load is low. Method No. 2 uses 0.1 cm thick layers of any of the standard adsorbents on glass plates, prepared in the usual manner[5] or purchased ready coated, *e.g.* from Analtech, Merck or Schleicher and Schuell. The plates are developed as in method No. 1 by descending continuous elution, wick fed with solvent from the upper trough. The plates stand in narrow glass troughs from which eluted fractions may be pipetted or pumped out. The troughs are flushed out with solvent between fractions to limit mixing. All other relevant details are as described for method No. 1.

These methods can be varied in several useful ways. Rapid measurement of ultra-violet and infra-red spectra is facilitated by collecting fractions directly in cuvettes or on potassium bromide. With multicomponent systems requiring two-dimensional development to obtain good resolution, *e.g.* protein hydrolysates, the chromatogram may be divided into strips after the first development run and the strips turned through 90° and treated as above in order to obtain the individual components.

This work was part of a programme on the development of the new histochemical methods supported by grants to the Department from the S.R.C. Thanks are also due to Unilever Ltd. for financial assistance.

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Received July 11th, 1968