A SIMPLE DEVICE FOR CONTINUOUS ELUTION IN FILM CHROMATOGRAPHY

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INTRODUCTION

In spite of the excellent results that can be obtained by normal film chromatography, there are situations in which a single development of a chromatogram does not give satisfactory resolution of the components of a mixture. To improve the resolution, two equivalent methods are available. In the first, the process of multiple development, the chromatogram is developed in the normal way. When the solvent reaches a pre-determined level on the film, the chromatoplate (or strip) is withdrawn from the developing tank, and after the solvent has evaporated from the film, the chromatogram is again developed in the normal manner. The process may be repeated as often as is necessary to obtain a satisfactory result. Equations for determining the optimum number of developments have been given by TRUTER[1].

The technique of multiple development is tedious and less convenient to operate than the alternative process, namely continuous development. Unfortunately, to subject a film chromatogram to continuous development, special apparatus is required. In those pieces of equipment that have been described hitherto, the direction of solvent-flow is abnormal. Film chromatograms are normally developed by the ascending-solvent technique. In the devices designed by STANLEY AND VANNIER[2], by MISTRYUKOV[3] and by REISERT AND SCHUMACHER[4] the solvent-flow is downward, and in the devices described by MOTTIER[5] and by BRENNER AND NIEDERWIESER[6] it is horizontal. As a consequence of the abnormal direction of solvent-flow, special arrangements are required for feeding the solvent on to the film.

To maintain a continuous flow of liquid through the film, the solvent must be removed when it reaches the further limit of the adsorbent. In the descending-development technique the solvent is allowed to drip off the chromatogram whereas in the horizontal-development technique the solvent is allowed to evaporate when it reaches the far edge of the adsorbent. Hitherto, no simple and satisfactory method for continuous development by the ascending-solvent technique has been described. If a chromatogram is developed in an open tank, the results are unsatisfactory because the solvent evaporates from the entire surface of the chromatogram; for satisfactory results, it is absolutely essential to limit evaporation of the solvent to a strip of adsorbent which is not part of the working area. In the apparatus described here, continuous development is obtained by allowing the solvent to ascend the film which projects through a slot in the lid of the tank. The dimensions of the slot are such as to restrict evaporation to that part of the adsorbent which is outside the tank.

Besides permitting continuous development to be carried out very simply, the slotted lid can also be employed as an auxiliary in increasing the resolution in a single-stage development. In general, the smaller the spot of sample at the origin, the better will be the resolution. The slotted lid may be used for converting a relatively large spot of sample placed at the origin into a line about 0.5 mm wide at the opposite side of the film.

EXPERIMENTAL

Apparatus

The process described here requires a developing tank from which the strip or plate will project by 1–2 cm when placed vertically in it. Other dimensions of the tank should be the minimum required to hold the plate. The lid is the important feature. For the development of chromatostrips, the lid is in two parts; one is a specially shaped piece of brass and the other is merely a glass cover for the remainder of the tank. For standard plates it is more convenient to use a machined brass strip and two glass plates as the lid. The essential features of the design of the brass strip are shown in Fig. 1. The dimensions of the brass strip which are not shown must be adjusted to

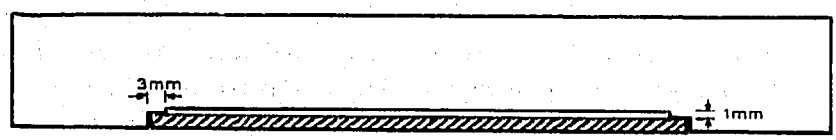


Fig. 1. Plan of the machined brass strip which is 6 mm thick. The hatched area represents the section of the chromatoplate.

suit the developing tank and the glass plate available so that, when the apparatus is assembled, the chromatoplate is effectively sealed all round except for the small gap facing the adsorbent film. When the brass is 6 mm thick, a gap of about 1 mm between the adsorbent surface and the recess in the brass strip is satisfactory; if thinner sheets of brass are used, the gap must be correspondingly smaller. During development of the chromatogram, the solvent climbs up the film and when it reaches the exposed part of the adsorbent outside the tank, it evaporates, and so continuous development of a vertical chromatogram is achieved.

Methods

A solution of a commercial dye, Oil Red OS, in methylene chloride was spotted on to a chromatoplate which had been prepared in the conventional manner using silicagel G (film thickness: 250 μm). The charged plate was then placed in the tank with the adsorbent-coated surface of the glass facing the brass strip and "clamped" by closing up to the other portions of the lid. Carbon tetrachloride was used as the developing solvent and the sides of the tank were lined with filter-paper soaked in solvent.

Formation of a hair-line origin was carried out as follows. A line sample of a solution of Oil Red OS was placed on the adsorbent in the usual way, the charged plate was placed in the developing tank, the slotted-lid was assembled and the chromatogram was "developed" in chloroform–methanol (10:1). After about 1 h the dye sample had been transferred to the opposite end of the chromatoplate where it formed a line about 0.3 mm in width. The plate was then withdrawn from the tank and after the solvent had evaporated from the film, the adsorbent was reactivated by heating

the chromatoplate to 110° for 5 min. Subsequently, the chromatogram was developed from the hair-line origin, using carbon tetrachloride as the solvent, by the continuous technique already described (Fig. 2); the hair-line origin is still clearly marked by the most polar fraction.

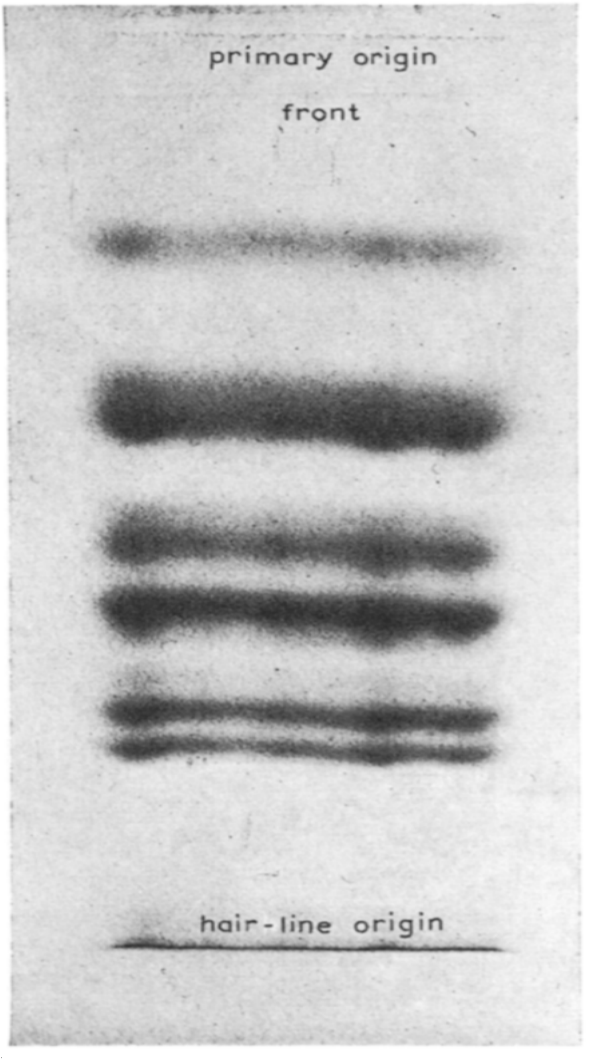


Fig. 2. One-dimensional continuous development of Oil Red OS from a hair-line origin. Adsorbent: silicagel G. Solvent: carbon tetrachloride. Time: 18 h. Distance from origin to front: 15 cm.

The chromatogram shown in Fig. 3 was prepared in a similar manner. After development in the first direction in carbon tetrachloride, the sample was compressed into a second hair-line origin at right angles to the first origin by development in chloroform–methanol (10:1). Subsequent treatment of the chromatogram was exactly as described above.

RESULTS AND DISCUSSION

For the formation of a hair-line origin a good solvent, which carries the sample with or near the solvent front, is required. When the solvent level reaches the upper edge of the slot, it evaporates and the sample is gradually deposited in the form of a line at the edge of the solvent front. With colourless samples it is important to ensure that all

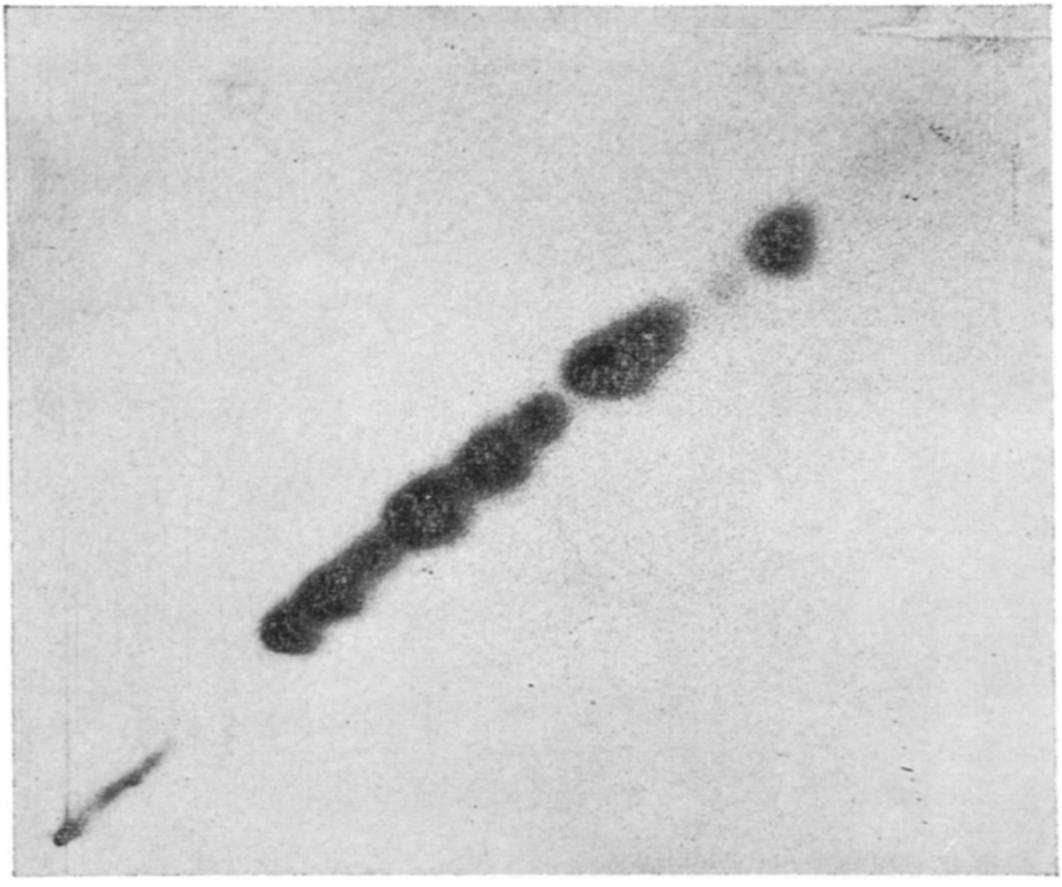
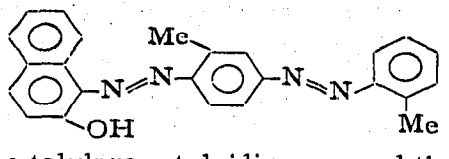


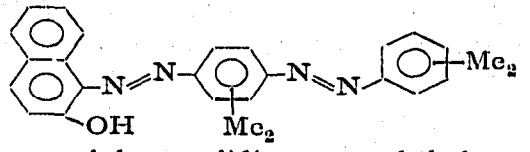
Fig. 3. Two-dimensional continuous development of Oil Red OS. Adsorbent: silicagel G. Solvent: carbon tetrachloride. Time: 16 h in each direction. Distance from origin to front: 16 cm.

the material has reached the front; no difficulty is encountered if the process is allowed to continue for longer than the minimum time. If a solvent which can de-activate the adsorbent has been used to produce the hair-line origin, *e.g.*, one containing methanol, the adsorbent should be reactivated by a brief thermal treatment. Under normal laboratory conditions hydroxylic solvents alone are not suitable for preparing a hair-line origin because they do not evaporate quickly enough; they form rather irregular fronts *above* the level of the slot.

In two-dimensional work the hair-line origin may be used for either or both developments. Fig. 3 shows an overloaded (total load: 2.4 mg) two-dimensional chromatogram in which the hair-line origin was used for both directions. The test sample, Oil Red OS, is a mixture of:



4-*o*-tolylazo-*o*-toluidine→2-naphthol and



xylylazo-xylidine→2-naphthol

As all the positional isomers of xylene are present in the starting material, the product would be expected to be a complex mixture of isomers. Fig. 3 shows that the mixture has been resolved into fourteen fractions and on the original chromatogram an addi-

tional four faint spots could be detected. Normal chromatography of the dye, using either benzene or methylene chloride as the developing solvent, failed to resolve the mixture into more than two fractions.

SUMMARY

A simple, inexpensive slotted lid which enables a film chromatogram to be developed continuously, and its application for transferring a sample to a hair-line origin, is described.

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