USES OF SHEET PARAFFIN AND PLASTIC FILMS IN PAPER CHROMATOGRAPHY: SPOTTING OF LARGE VOLUMES ON PAPER; ELUTION; STRIP CHROMATOGRAPHY

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The maintenance of a saturated atmosphere and the spotting of large volumes as small spots have always presented technical problems in the use of paper chromatography. The following uses of sheet paraffin (Parafilm**) as an adhesive vapor barrier have been found to greatly simplify the manipulations, and without the use of cumbersome containers or elaborate equipment. For use with solvents or solutes which dissolve or interact with paraffin, polyethylene or other suitably inert sheet material may be substituted for the paraffin in an appropriate manner.

For spotting of large volumes on paper

A paper wick is attached to a glass funnel (Fig. 1) by means of sheet paraffin, to produce a steady capillary flow onto the paper sheet at any desired rate. Any convenient volume of solution may be applied depending on the size of the funnel used. A 35-mm top chemical funnel with 3-mm inside diameter stem cut off to 15-mm length will conveniently dispense 0.05 to 5 ml of solution and is used by way of illustration.

The rate of descent of solution is governed by the width of wick of filter paper (as example, a 1 × 30-mm strip) passing through the stem of the funnel. Sheet paraffin of such size (a 20 × 40-mm rectangle) as to protrude below the funnel stem by about 1 cm is evenly wrapped around the funnel stem with moderate pressure (Fig. 1,A), then is flattened and crimped by finger pressure below the end of the stem to seal off the bottom of the stem, with wick passing through as shown in Fig. 1,B. Cutting the protruding wick fairly short prevents excessive curling and facilitates exact positioning of the spot. Polyethylene film[1] may be substituted for paraffin if required. It is wrapped around the stem, smoothly sealed with cellophane tape along the outer seam and upper edge (out of contact with solvent), then heat-sealed at the

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bottom, with wick passing through. A removable extension (Fig. 1,C) of the same outer diameter as the funnel stem is an aid to evenly rolling and taping the protruding portion of polyethylene.

The result is a rigid, essentially transparent dispensing funnel whose rate of flow can be regulated over a wide range by the size and type of paper used for the wick. The rate of flow chosen will depend entirely on the drying rate available and

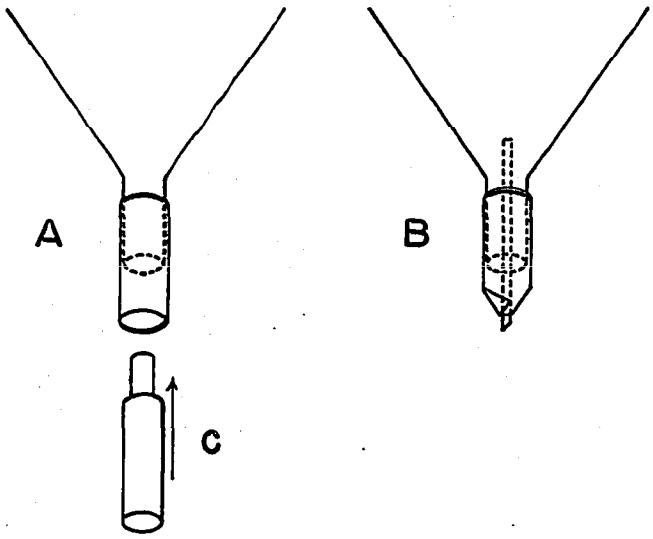

Fig. 1. Funnel. A: Stem wrapped in parafilm sheet. B: Crimping of paraffin around paper wick. C: Stem extension, removed.

the size of spot desired. Spots of small area may be obtained at rates of less than 0.1 to several ml per hour, dependent on the solvent used, temperature of the air stream, etc. The funnel is emptied completely and may be quantitatively rinsed with a few drops of solvent. It can be re-used indefinitely after rinsing; or it can be discarded and reassembled as needed since its construction is so simple.

Support of the funnel, or a battery of funnels, can be simple or elaborate, corresponding to the need. (Ring stands and bulb-leveling supports with screw-type height adjustment can be conveniently used.) Once the funnel, covered by a watch glass to

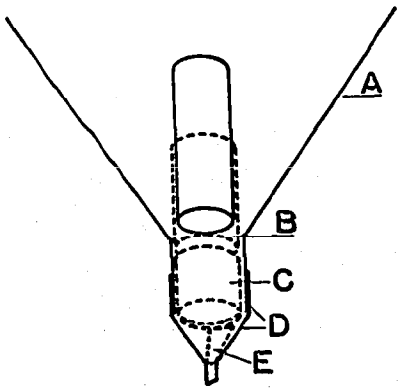

Fig. 2. Elution chamber. A: Powder funnel. B: Paper piston. C: Paper to be eluted. D: Paraffin seal. E: Paper wick.

minimize evaporation, is positioned, no further attention is necessary. The funnel shape lends itself to the use of cooling coils if desired for unstable solutions.

For elution and respotting as a single operation

The same wick-controlled flow may be used at the lower end of an elution chamber such as has been described by CANNY[2], or a simpler modification thereof, when the eluted solution is to be respotted on paper. Thus, a large number of tedious intermediate manipulations are eliminated. A simplified form of elution chamber (Fig. 2) is assembled from a laboratory powder funnel (A) with stem size such as to accommodate the paper to be eluted. A paper wick (E) passes through the paraffin-sealed lower end of the stem (D), as described. A piston-like loosely fitted glass rod or small test tube, wrapped in a 2- to 3-layer roll of filter paper (B) to make a snug fit, enters from the top of the stem. The paper to be eluted (C) is rolled on forceps to fit within the funnel stem in such a way as to be in contact with the wick at the lower end and with the piston-like paper roll at the top end when the latter is pushed into the stem. The wick used for elution is preferably cut broadened where it extends above the paraffin seal, in order to make better contact with the eluted paper. Under some circumstances, of course, two or all three paper components of the system (piston, eluted paper, and wick) may be cut out as a single piece of paper, avoiding problems of contact. A volume of solvent added to the funnel will elute the paper by capillary flow. As previously, the rate of flow onto the paper being spotted can be controlled by the size of wick used.

For collection of eluted solution, or development of chromatographs

A paraffin jacket also may be used to avoid evaporation during a conventional paper strip elution, where eluate is collected in a container. Two oversize paraffin sheets are cut, the paper is laid between them as shown in Fig. 3, and pressure is evenly applied to cover the strip of paper smoothly on both sides and to seal the edges (as well as to join two or more strips of paper). The upper portion of the paper, not covered

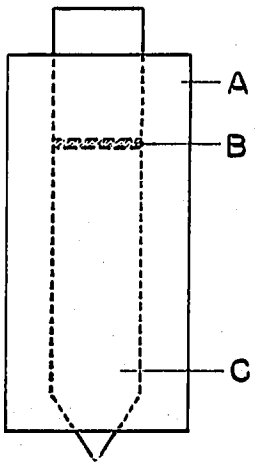

Fig. 3. Jacketing of a paper strip. A: Paraffin jacket (2 sheets). B: Illustration of joining of paper strips (by overlapping the papers). C: Paper to be eluted.

by paraffin, is dipped into a solvent trough, or a solvent reservoir fashioned from paraffin[3] is used. Polyethylene may again be substituted. The jacketing technique for producing a self-contained saturated atmosphere may be used in similar manner in many applications of both descending and ascending paper strip chromatography. Clumsy containers to maintain a saturated atmosphere are thereby simply eliminated, many solvents may be used concurrently with little inconvenience, and the compact strips may be processed on any convenient laboratory bench. A thin jacket is particularly useful with radioactive compounds in permitting the location of radioactivity during its migration on the paper.

SUMMARY

A number of simple chromatographic applications are described in which sheet paraffin or other plastic film is used as an adhesive vapor barrier:

(1) A funnel attached to a paraffin-enclosed narrow paper wick for spotting large volumes of sample solution as small spots of controlled size.

(2) A funnel for the elution of a spot and its reapplication to another paper in a single operation.

(3) A method for elution or chromatography which requires no jars or other rigid saturation chambers.

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