

## XI. CHROMATOGRAPHIC APPARATUS

Chromatography, a powerful analytical technique of recent development, may be performed with relatively simple apparatus. It is based upon the differential migration of solutes in a liquid or solid medium and maybe used for both qualitative and quantitative analysis of solutions.

### A. QUALITATIVE CHROMATOGRAPHIC APPARATUS

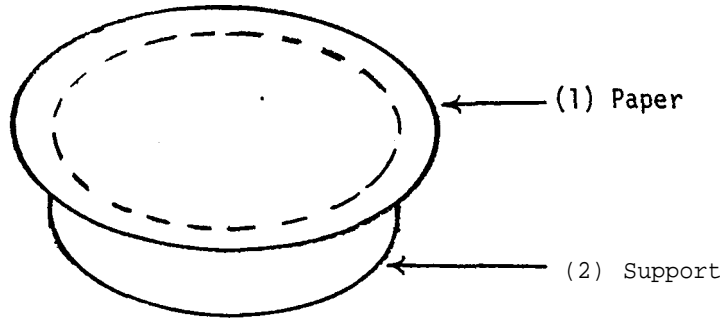
This section includes chranatographic devices employing paper as the stationary medium and briefly describes a few techniques for using these devices to identify the components of a mixture.

### B. QUANTITATIVE CHROMATOGRAPHIC EQUIPMENT

This section describes a device that allows for the separation of the components of a mixture as well as the recovery of individual components for further experimentation or purification.

A. QUALITATIVE CHROMATOGRAPHIC APPARATUS

A1. Horizontal Paper Chromatography Device



a. Materials Required

<u>Components</u>	<u>Qu</u>	<u>Items Required</u>	<u>Dimensions</u>
(1) Paper'	1	Filter Paper (A)	Approximately 10 cm diameter or larger
(2) Support	1	Petri Dish (B)	Slightly smaller than filter paper

b. Construction

(1) Paper

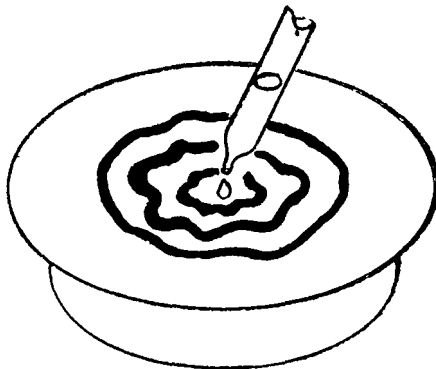
Use a circle or square of filter paper (A) as the medium for the chromatogram.

(2) Support

Select a petri dish or other shallow container (B) just slightly smaller than the paper (A) on the support (B).

c. Notes

(i) This apparatus can be set up almost instantaneously for rapid, qualitative work. A drop of a colored solution to be analyzed is placed in the center of the paper. Then, successive, small drops of the eluting solvent are dropped on top

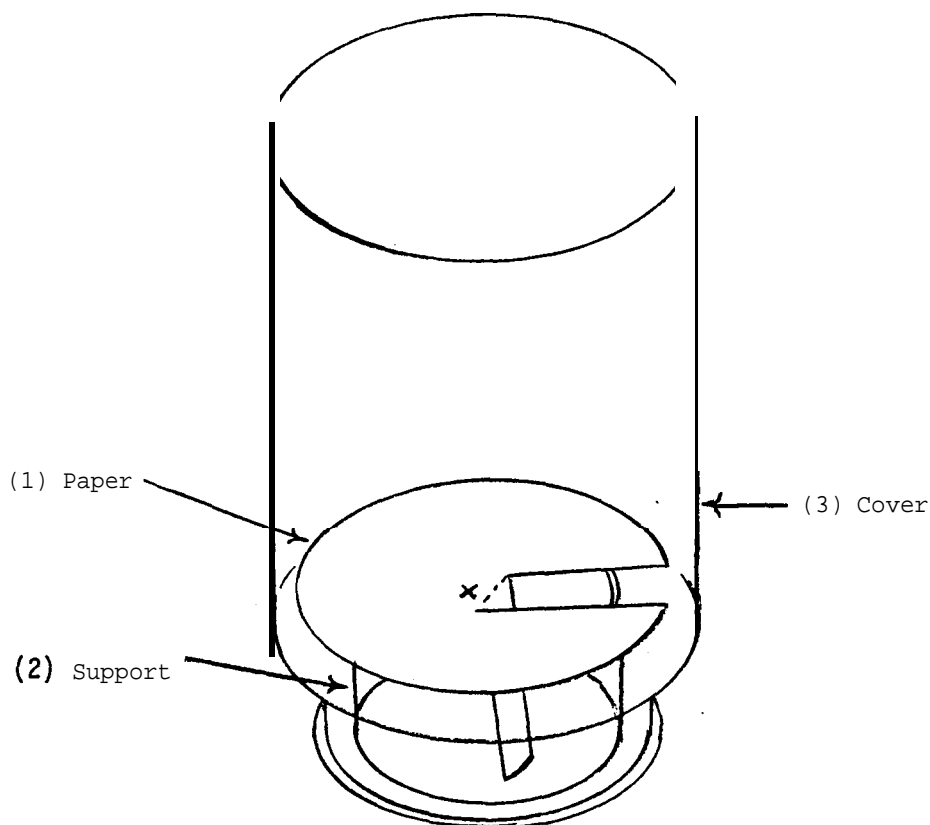


of the original drop. The solution spreads radially, and as separation of components occurs, concentric rings of color will appear on the paper.

(ii) As an example of a test solution, a drop of black or blue-black, washable ink may be used. The eluting solvent in this case could be water, methanol (methylated spirits) or 70% ethanol.

(iii) Chromatography paper, white paper towels, blotting paper, newsprint paper, or other white or light-colored, coarse-grained paper may be substituted for the filter paper (A).

A2. Horizontal Paper Chromatography Device \*



a. Materials Required

<u>Components</u>	<u>Qu</u>	<u>Items Required</u>	<u>Dimensions</u>
(1) Paper	1	Filter Paper (A)	Approximately 10 cm diameter or larger
(2) Support	1	Cup or Jar (B)	Slightly smaller than filter paper
(3) Cover	1	Glass Jar or Bowl (C)	To cover paper and support

b. Construction

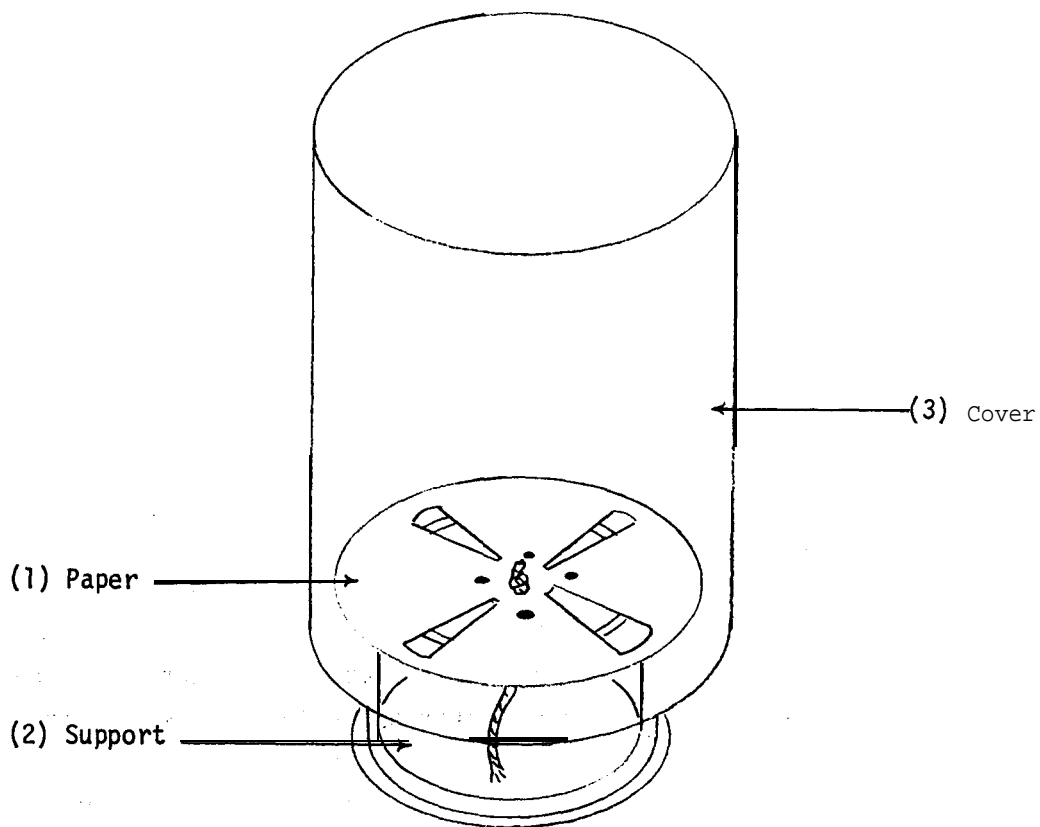
(1) Paper

Take a circle or square of filter paper (A) or suitable substitute and cut a tongue across the paper to within about 1 cm of the center of the

\*Adapted from A. V. Jones, "Chromatography for Junior Schools," School Science Review, CLXXIX: (1970), 298-300.



A3. Horizontal Paper Chromatography Device \*

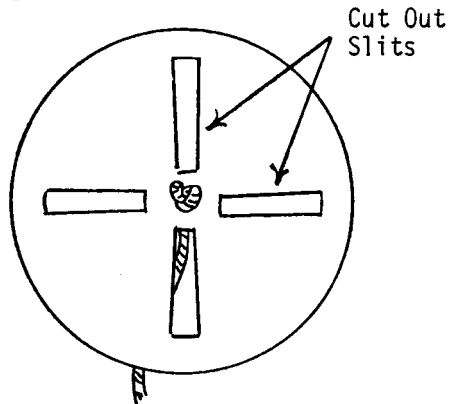


a. Materials Required

<u>Components</u>	<u>Qu</u>	<u>Items Required</u>	<u>Dimensions</u>
(1) Paper	1	Filter Paper (A)	Approximately 10 cm diameter or larger
	1	String (B)	Approximately 0.2 cm diameter, 5-10 cm long
(2) Support	1	Cup or Jar (C)	Slightly smaller than filter paper (A)
(3) Cover	1	Glass Bowl or Jar (D)	To cover paper (A) and support (C)

b. Construction

(1) Paper



(2) Support

(3) Cover

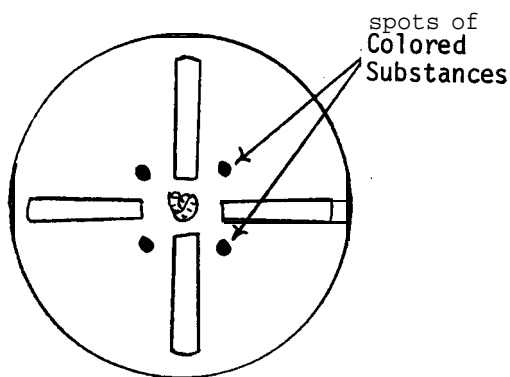
Take a circle or square of filter paper (A) or suitable substitute and cut several slits radiating from the center as shown. Punch a small hole in the center and secure a piece of string (B) with a knot, to act as a wick.

Select a small cup or jar (C) just slightly smaller than the paper (A). Rest the paper (A) on the rim of the jar (B) so that the string wick (C) extends into the jar.

Select a large glass jar or bowl (D) to cover the support and paper. Invert the cover to enclose the other components.

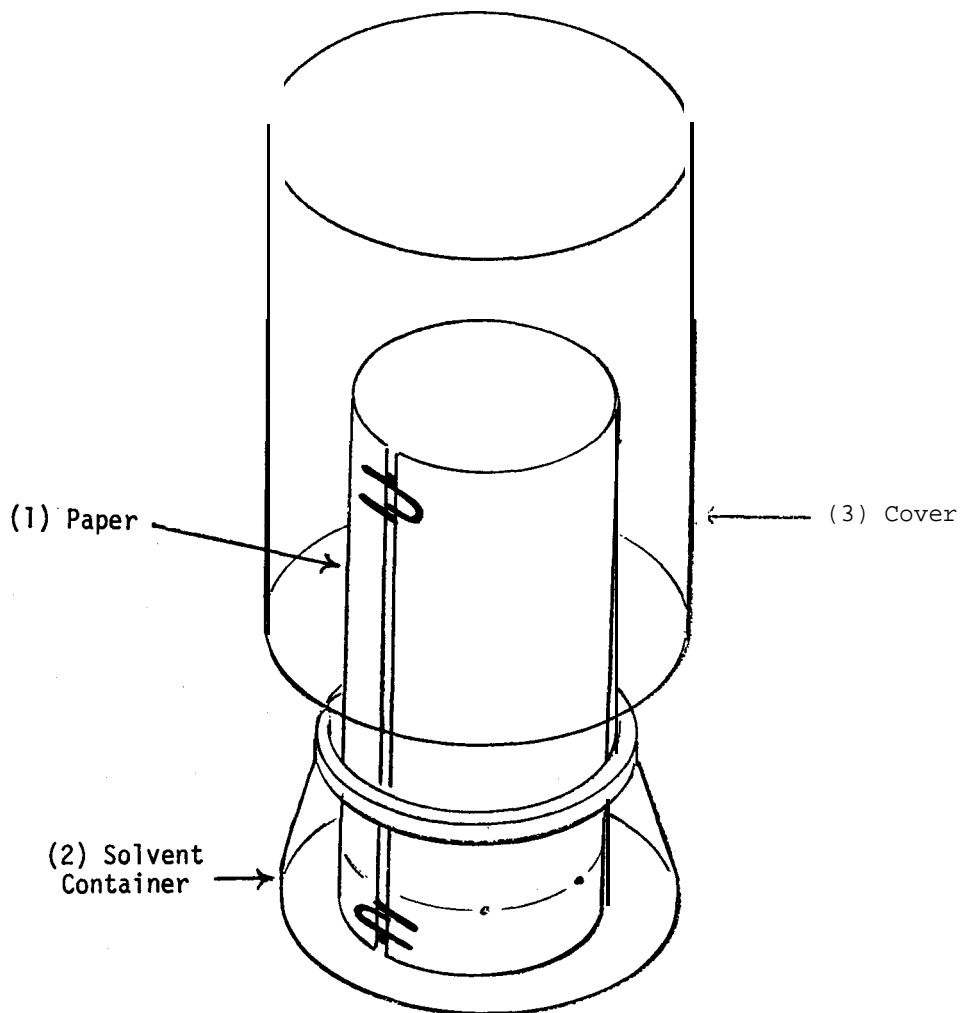
C. Notes

(i) This apparatus is used in the same fashion as the preceding device. However,



ever, the slits in the paper allow for more than one colored substance or test solution to be used simultaneously. The spots are placed inside the "V" of the slits, which prevent the colors from merging.

A4. Vertical Paper Chromatography Equipment



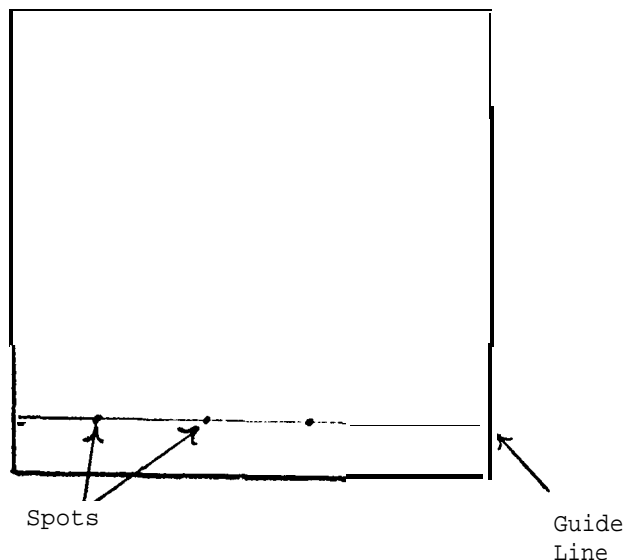
a. Materials Required

<u>Components</u>	<u>Qu</u>	<u>Items Required</u>	<u>Dimensions</u>
(1) Paper	1	Chromatography Paper (A)	Approximately 15 cm x 15 cm
(2) Solvent	1	Beaker, Bowl or Jar (C)	To contain paper (A) when rolled into tube
(3) Cover	1	Glass Jar or Bowl (D)	To fit on or over Solvent Container (C)

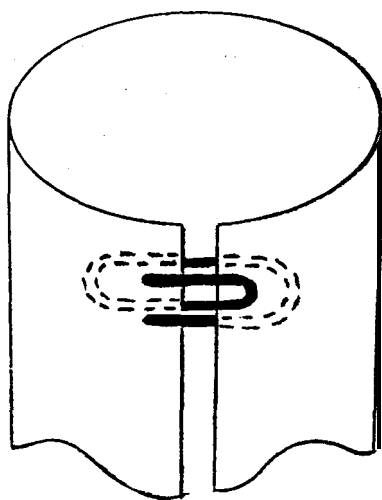


b. Construction

(1) Paper



With a pencil, draw a faint line approximately 2 - 3 cm from one edge of the paper (A). Use this line as a guide for locating the spots of solution or solutions to be tested. Make the spots as small as possible and about 2 cm apart.



Roll the paper (A) loosely into a tube. Secure the edges together with the paper clips (B) such that the edges do not touch or overlap.

(2) Solvent Container

Set the rolled paper (A) into the beaker, bowl, or jar (C) and pour solvent into the container (C) to a height of about 1 cm.

(3) Cover

Rest a large glass jar or bowl (D) on or over the solvent container (C) to prevent evaporation of the solvent.

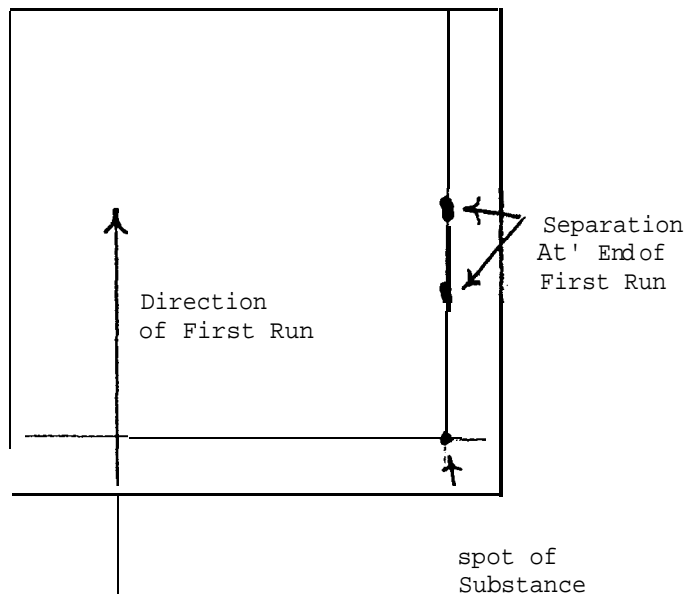
c. Notes

(i) If chromatography paper is not available, white paper toweling or blotting paper may be substituted.

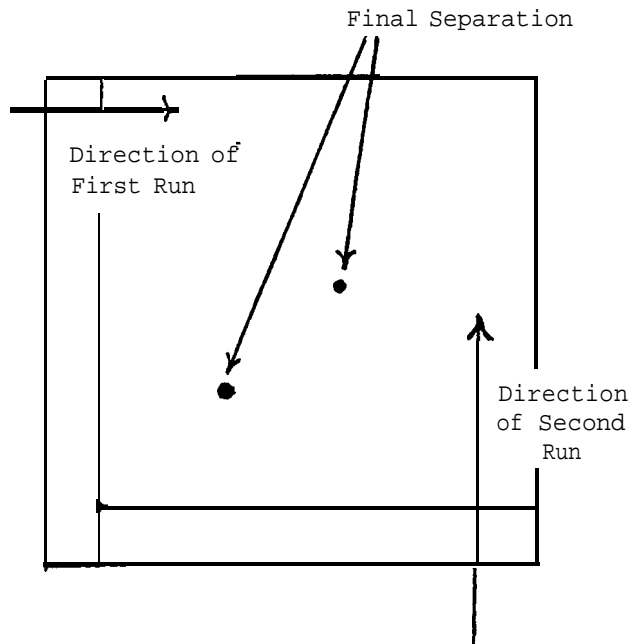
(ii) When this apparatus is in use, the solvent front migrates up the paper, (by capillary action) resulting in the separation of the components of the test spot. This is allowed to continue until it has reached to within several cm of the top of the paper or until it is apparent that the solvent front will move no further (when the rate of capillary action is in equilibrium with the rate of evaporation). The paper is then removed from the apparatus and dried, and the final locations of the color spots may be circled with pencil for easy identification.

(iii) This apparatus is also suitable for performing separation of colorless substances, as long as the completed chromatogram can be treated in some way to make visible the final location of the component of the substances. For example, proteins, while generally colorless, may separate in this fashion. The dried chromatogram is then sprayed with a ninhydrin solution, which reacts with the amino acids in their final locations, making them visible as bluish spots or smudges.

(iv) It is possible, with this apparatus, to submit a substance to chromatographic separation by two different solvents on the same sheet of paper. To run



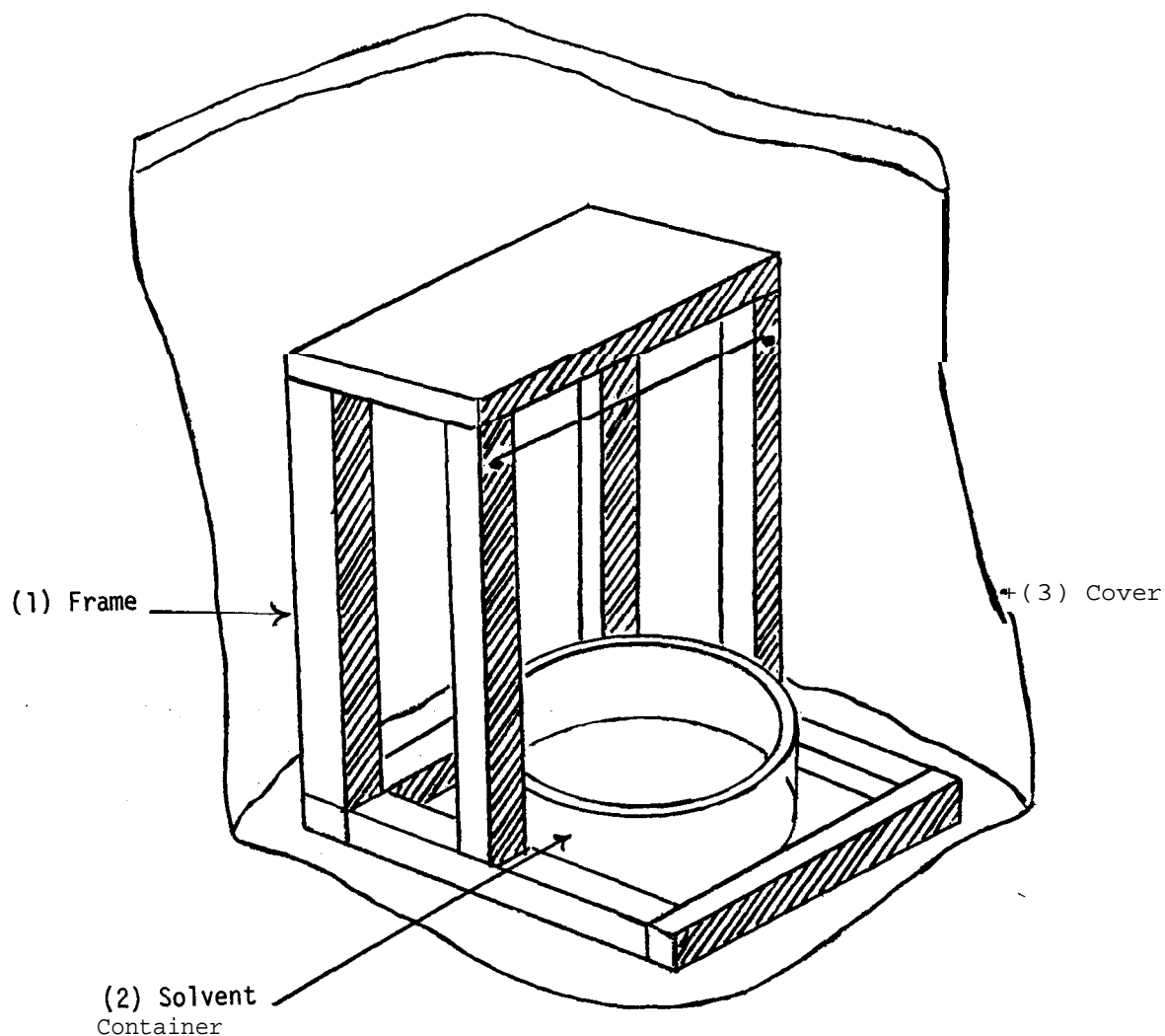
such a two-dimensional chromatogram, a spot of the substance is placed at the intersection of two lines drawn on the paper and treated as described above. with one solvent. At the end of the first run, the chromatogram is removed from the apparatus and dried.



Then the paper is rotated  $90^\circ$  and again rolled into a tube, with the first separation at the bottom edge of the tube. This tube is run a second time with a second solvent. Thus, it is possible to effect a more complete separation than is possible with one solvent alone.

(v) A complete discussion of techniques and substances appropriate to chromatographic separation is beyond the scope of this guidebook. For further information, texts and resources on biochemistry, chemistry, and chromatography should be consulted.

A5. Vertical Strip Paper Chromatography Equipment

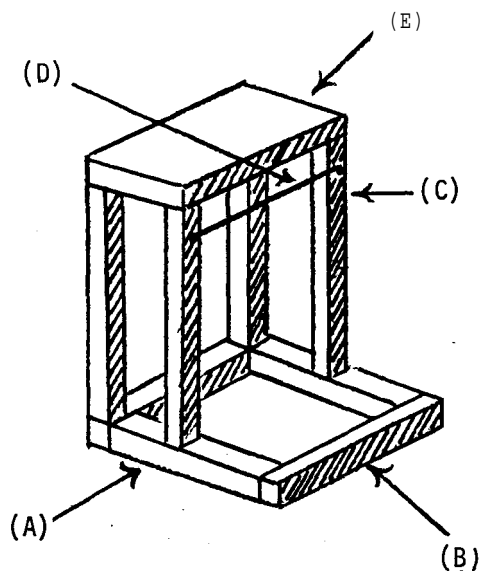


a. Materials Required

<u>Components</u>	<u>Qu</u>	<u>Items Required</u>	<u>Dimensions</u>
(1) Frame	2	Wood (A)	1 cm x 1 cm x 8 cm
	2	Wood (B)	1 cm x 1 cm x 10 cm
	4	Wood (C)	1 cm x 1 cm x 20 cm
	1	Wood or Masonite (D)	6 cm x 10 cm x 0.2 cm
	1	Thin, Stiff Wire (E)	Approximately 11 cm long
(2) Solvent	1	Cup or Jar (F)	Approximately 4 cm high, to fit inside frame
(3) Cover	1	Plastic Bag (G)	To fit loosely over frame

b. Construction

(1) Frame



With nails and glue, secure the frame parts (A), (B), (C), and (D) as shown. Secure the wire (E) to the frame, about 2 cm from the top, with two small nails.

(2) Solvent Container

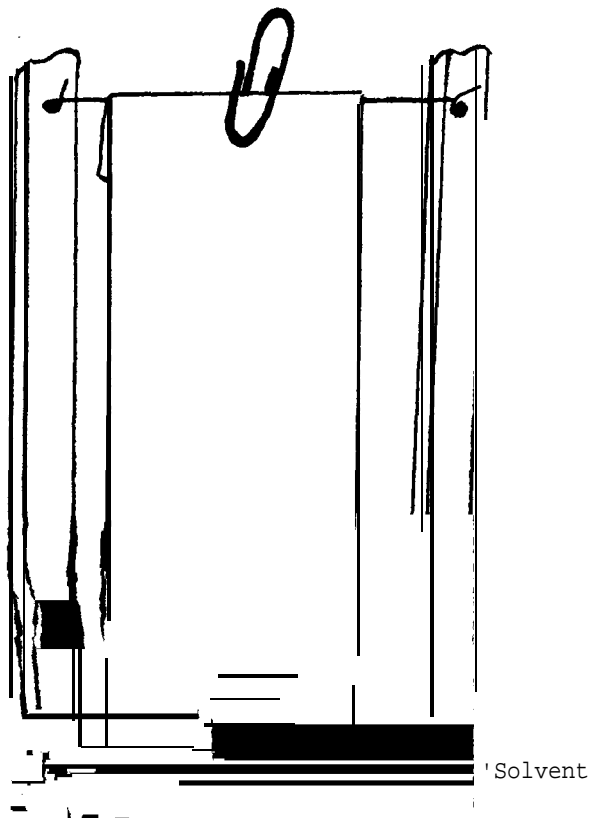
Select a shallow cup or jar (F) that will fit inside the frame. If necessary, cut a tall jar down to a height of 3 - 4 cm (I/F2).

(3) Cover

Select a plastic bag (G) that will fit loosely over the frame. It may be held in place by clipping it with a clothespin to a clamp or ring that is supported about 10 cm above the frame on the ring and burette stand (IV/B5) or other suitable support. Alternatively, a frame to support the bag may be constructed out of stiff (e.g., coat hanger) wire.

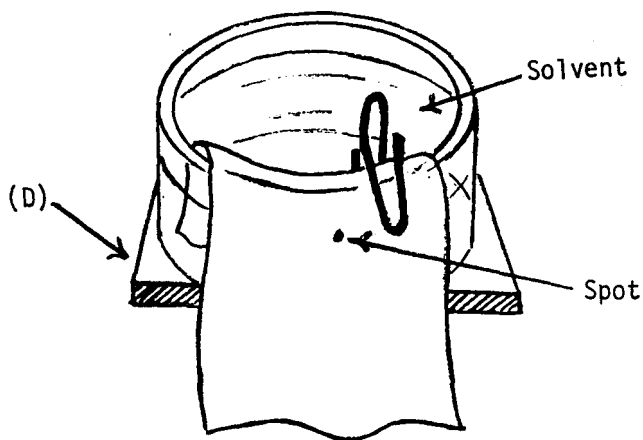
c. Notes

(i) This frame may be used to support a strip of chromatography paper or suitable substitute for either ascending or descending chromatographic operations.



For ascending operations, the solvent container (F) remains at the bottom of the frame. The Paper strip, with one end just touching the solvent, is hung from the wire with a paperclip. The spot or spots of substance to be separated is located at the lower end of the strip, just above the solvent. The apparatus should be kept covered by the plastic bag (G) during the course of the experiment to keep solvent evaporation to a minimum.

(ii) In order to use the frame for descending operations, the solvent container (F) is placed on the top shelf (D).

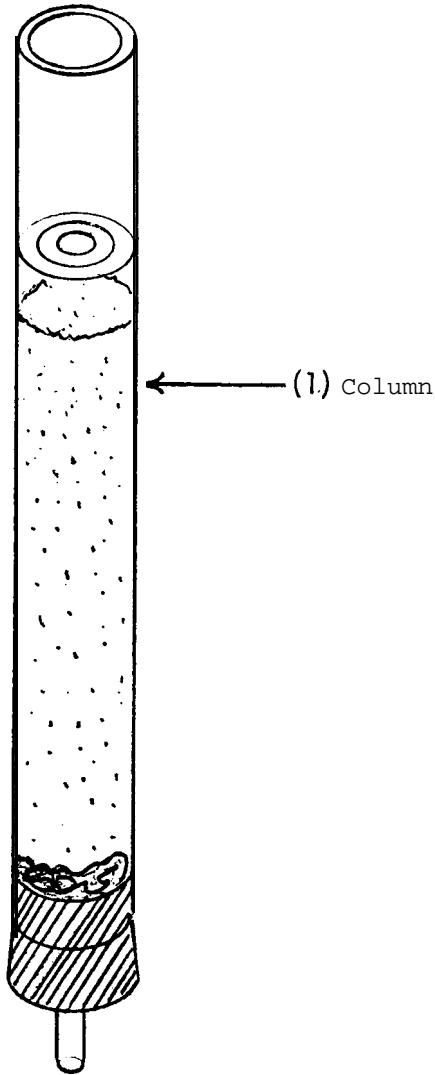


the solvent container, held in place with a paper clip or clothespin, and with a short piece folded over to dip into the solvent. The spot is located near the top of the strip, outside the solvent container. The solvent front then moves down the paper in the course of the experiment.

(iii) If a sufficiently large jar is available, it may be used as a cover in place of the plastic bag (G).

B. QUANTITATIVE CHROMATOGRAPHIC EQUIPMENT

B1. Liquid-Column Apparatus

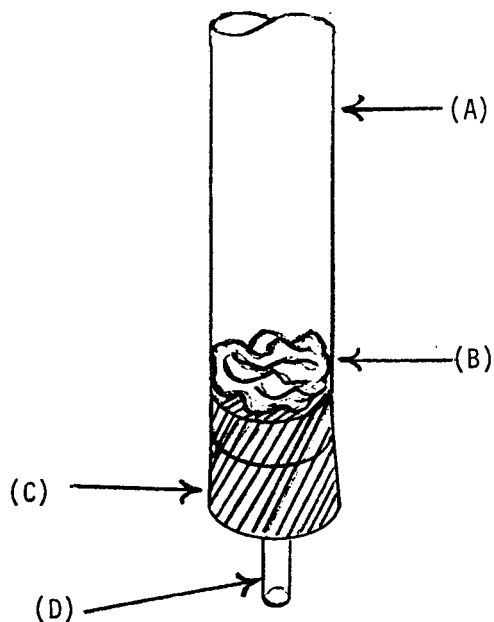


a. Materials Required

<u>Components</u>	<u>Qu</u>	<u>Items Required</u>	<u>Dimensions</u>
(1) Column	1	Glass Tube (A)	Approximately 1.5 cm outside diameter, 25 cm long
	1	Cotton or Glass Wool (B)	--
	1	1-Hole Stopper (C)	To fit tube
	1	Glass Tubing (D)	Approximately 0.5 cm diameter, 5-10 cm long
		Silica Gel (E)	28-200 mesh

b. Construction

(1) Column



Fire polish both ends of the glass tube (A) to eliminate sharp edges. Push a small wad of cotton or glass wool (B) about 1 cm into one end as a plug.

Insert the small glass tube (D) into the stopper (C) and push the stopper into the large glass tube (A), and support the column in a vertical position in a burette clamp (IV/B5) or other suitable support.

To pack the column with the stationary medium, make a slurry with several grams of the silica gel (E) and water. Pour this slurry into the top of the column, and allow the water to drain through the small glass tube (D), while the moist silica gel is retained by the plug (B). If necessary, pour additional slurry into the column until about 15 cm of the column is packed with silica gel and about 1 cm of water remains on top of the silica gel. If desired, the packing operation may be hastened by applying slight suction, by means of the suction-filter flask (VI/A4) coupled with a suction pump, aspirator (V/A8) or other source of suction.

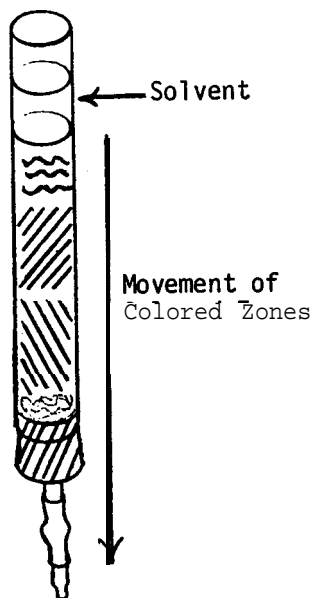


c. Notes

(i) The flow of liquid through the column may be controlled, if desired, by the addition of a stopcock, or flexible rubber tubing coupled with a pinch clamp (IV/A4) or glass bead (III/B1). A glass nozzle may also be added to the free end of the flexible tubing.

(ii) To use this apparatus, the water remaining on the column is allowed to drain until less than 1 cm remains to cover the silica gel. Then a small quantity of a solution of colored material to be tested, in a concentrated form, is gently pipetted on to the medium. The desired solvent is then added to the column, and the column is allowed to drain slowly, using either gravity or very slight suction.

As the solvent moves down the column, carrying the substance with it, separation will occur, as indicated by colored zones appearing on the medium. As



additional solvent is added to the column, the zones themselves will migrate down the column; if sufficient solvent is added, each zone, consisting of a specific component of the substance tested, may be washed off the column and recovered separately.

(iii) In addition to separating components of a substance and washing them down the column with one solvent, it is possible to use additional solvents to wash down a component or components that do not migrate at all with the first solvent. To do this, allow the column to drain until less than 1 cm of the first solvent remains on top of the medium, then add the second solvent to the column and proceed with the washing as described above.

(iv) Other interesting results may be obtained by reversing the order of solvents used, in successive runs, with the same test substance. For example, alcohol and water are two solvents that may be used, in either order, to separate a mixture of vegetable dyes or ink.

(v) One of the chief advantages of the liquid-column method of chromatographic

separation over paper chromatography is that the components of the substance tested are recovered individually for use in further experiments or in quantitative determinations. For example, a measured quantity of the test substance, in a known concentration, is added to the column, and the solvent used and the solutions recovered are measured. Then the components eluted are submitted to volumetric or gravimetric quantitative analysis to determine the proportion of each component present in the original sample.

(vi) Substances other than 28 - 200 mesh silica gel, and solvents other than alcohol or water, may be used in liquid-column chromatography. Further experimentation, as well as research into the technical literature on chromatography, is suggested for the development of this technique. A useful reference for this purpose is Erich Heftmann, Chromatography, Second Edition, (New York: Reinhold Publishing Corporation, 1967).