An Improved Apparatus and Procedure for Ascending Paper Chromatography on Large Size Filter Paper Sheets

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The technique of ascending paper chromatography introduced by Williams and Kirby (*5*) represents a considerable simplification of the original technique of Consden, Gordon and Martin (*1*). However, the apparatus suggested by Williams and Kirby limits the possible size of two-dimensional chromatograms to about 11 in. × 11 in., and the actual chromatogram is usually considerably smaller when unused paper has been trimmed from the edges. The 6-gal stoneware crocks proposed, are quite heavy and unwieldy and become increasingly difficult to handle in larger sizes. Stoneware crocks have some other disadvantages: they are porous and tend to retain a particular solvent tenaciously; they are supplied with rounded edges difficult to seal properly unless a grinding operation is undertaken; and they have rounded inside bottoms which make it difficult to balance an ordinary flat-bottomed pan.

The apparatus to be described has made it possible to secure consistently good two-dimensional chromatograms by the ascending technique on 18 in. × 22 in. Whatman No. 1 filter paper sheets or on 23 in. × 23 in. S & S No. 604 filter paper sheets. The chromatography tank[3] itself is a 20-gal 26-lb stainless steel tank provided with two carrying handles, and having an inside height of 24 in. and inside diameter of 16 in. At the top, the wall is rolled outward to form a smooth flat flange, approximately ¾ in. wide. This flange acts as the lower surface of the lid-tank seal. The lid is a heavy plate glass disk to which a circle of soft rubber, approximately 1 in. in width, has been bonded by means of a Neoprene bond. The lid-flange junction provides a seal sufficiently good to permit a considerable reduction in the pressure within the tank without leakage, when suction is applied through an eccentrically placed 1-in. hole provided in the lid of the tank.[4] The eccentrically placed aperture provided in the lid is also useful for adding or removing material from solvent pans during a run, and may be used to introduce a thermostat and heating coil for temperature control.

Particular provision has been made for accurate leveling. Two spirit levels, separated by an angle of 90°, are permanently attached to the tank lid. The tank itself stands upon three legs, the length of which is individually adjustable. The leg length is sufficient to provide clearance for a spigot placed beneath the tank in order to drain flushing solutions or to drain solvent, when the entire tank base has been used as a solvent pan.

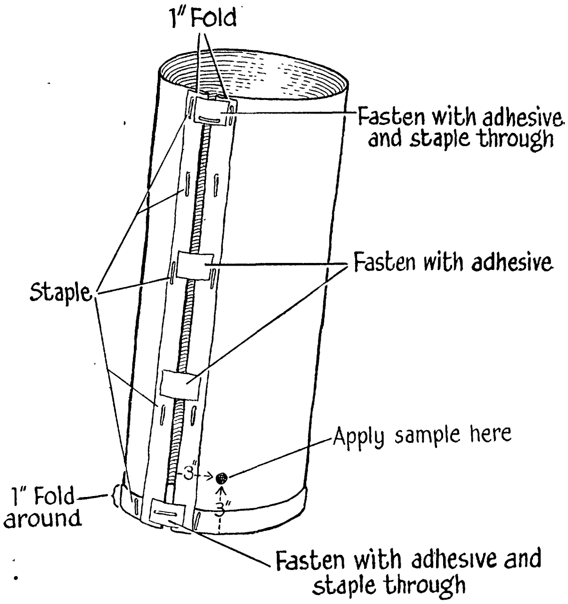

FIG. 1. Technique of folding and fastening large filter paper sheets. When the run is completed, the staples are carefully removed, and the adhesive tape cut. The sheet is hung for drying; and, if a two-dimensional chromatogram is contemplated, it is reformed into a cylinder and refastened after drying. At this time, excess adhesive from the first run is removed. Spots of material to be fractionated are best applied about 2 in. above the lower border and 2 in. in from the vertical border which is to be the lower border when the sheet is run in the second direction.

In general, the procedure used follows that of Williams and Kirby with few exceptions. Because the development of ascending chromatograms is slower than that of descending chromatograms, S & S No. 604, a more porous and softer paper than Whatman No. 1, is used under most circumstances and provides a considerably faster run than Whatman No. 1. The latter, however, should be used when solvents of high water content are employed, as in the protein experiments mentioned below, because of its stiffer quality.

The use of large paper sheets for ascending chromatograms requires a special technique to prevent bending or crumpling, since this will invariably occur if 18 in. × 22 in. or 23 in. × 23 in. sheets are merely stapled into a cylinder before standing in the solvent. Fig. 1 illustrates a technique of folding and fastening the borders of the cylinder which effectively overcomes the tendency to bend or crumple, even in solvents of high water content. The

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[1] We wish to thank Dr. Charles E. Dent of University College Hospital Medical School, London, for his advice on many points of chromatographic technique and for running the two-dimensional descending chromatograms in the case of cystinuria mentioned.

[2] This Department is in part supported by the Michael Reese Research Foundation.

[3] Photographs of the chromatography tanks are available upon request from the manufacturer. American Stainless Equipment Company, 2312 Greenview Avenue, Chicago, Ill.

[4] For runs with reduced tank pressure, a special stainless steel lid with a glass porthole is used.

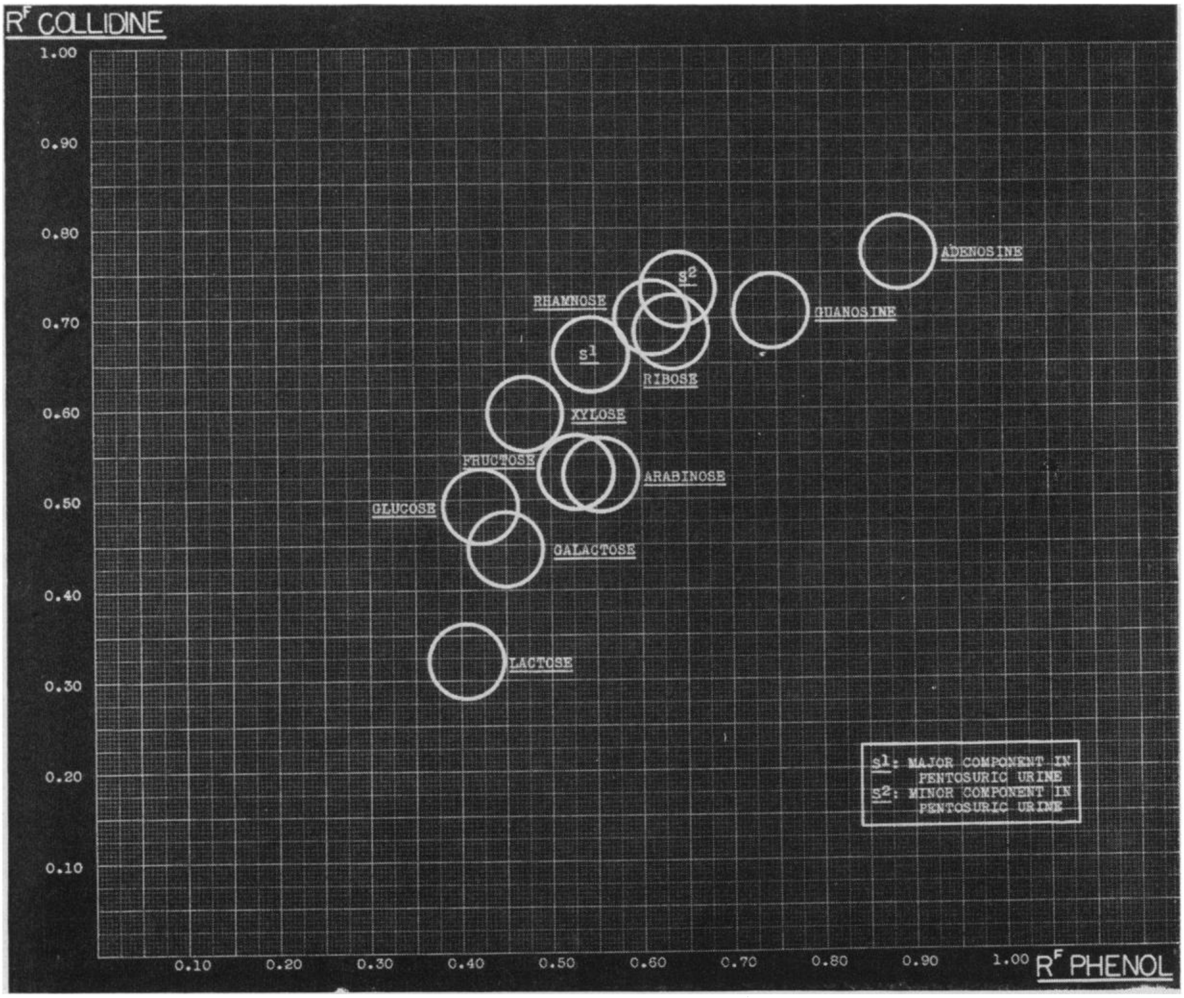

FIG. 2., Probable location of sugar spot centers on ascending chromatograms. The center of a particular sugar spot will fall in the circle in over two-thirds of the runs. Increasing temperature causes a rise in phenol R_f and a decrease in collidine R_f. To control such variation it is convenient to run a 1 µl spot of a standard solution, containing 5% rhamnose, xylose, arabinose, and galactose. The R_f of the unknown may then be corrected by multiplying the observed value by the fraction: theoretical average R_f of standard sugars ÷ observed average R_f of standard sugars.

staples used were ordinarily commercial staples; no difficulty attributable to metallic interference has been encountered. We have not noted the water-logging which Williams and Kirby found troublesome, possibly because all solvents, even when apparently free of emulsified or droplet water, are passed through a double thickness of large coarse fluted filter paper before being placed in the solvent pan, and refiltered if the filtrate is at all hazy. When this precaution is observed, ordinary water-saturated solvents give results which in our hands are superior to those obtained when using solvents saturated with saturated sodium chloride solution as recommended by Williams and Kirby (*5*).

Approximately 150 cc of solvent are placed in the solvent pan for a run on 23 in × 23 in paper. In the original technique of Consden, Gordon, and Martin (*1*), saturation of the atmosphere was maintained through the use of extra solvent-water and water-solvent pans. Such extra pans are not used in ascending chromatography, but a considerable excess of solvent is provided so that a saturated atmosphere may be obtained without significantly disturbing the concentration of the solvent with respect either to water or to solvent.

Large sheet ascending chromatography has been applied to a number of clinical problems with satisfactory results. In some respects, the technical quality of the ascending chromatograms exceeds that obtained by the descending technique, because of the virtual absence of tendency to develop irregular fronts.

Our R_f values for amino acids closely agree with those obtained by Williams and Kirby (*5*). R_f values for amino acids obtained by ascending and descending techniques are not directly parallel, although good parallelism obtains in the sugar series. Results obtained in the study of aminoaciduria by the two techniques have been identical. In a patient with cystinuria, both techniques detected the presence of excess amounts of lysine in each of two 24-hr urine samples with excess arginine in only one of the two samples. The apparatus and technique described have also been used in the partial development of a simplified procedure for the detection of purines on paper chromatograms (*2*).

Fig. 2 indicates the location of certain sugars of clinical interest on two-dimensional ascending chromatograms. A sample of urine from a patient with hereditary familial pentosuria yielded the two abnormal components, S^1 and S^2, shown on the grid. Studies to identify these components and a simplified procedure for the detection of sugars on paper chromatograms will be reported in a later communication (*7*).

The features of the tank design which permit operation at reduced pressures were introduced because of findings during preliminary attempts to fractionate protein mixtures and diazotized protein mixtures. In our experiments (*6*) and in those of Tiselius (*4*), it was found that when inorganic salt solutions were employed as developing solvents, no fractionation occurred unless water was permitted to evaporate from the solvent during the run. The results we obtained by casual evaporation did not prove sufficiently constant or precise for useful application; but by employing low, controlled vapor pressures, Tiselius (*4*) has obtained more promising results. Tiselius has also obtained distinct fractionation of protein mixtures on paper by a somewhat different technique, that of salting-out adsorption (*3*). In this procedure, the developing solvent is a salt solution which has a salt concentration approaching that at which the protein component of minimum solubility will precipitate.

References

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Electrical Still

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Many techniques, particularly the microdetermination of heavy metals, require metal-free water. Water from the usual steam tin-lined stills contains significant quantities of heavy metals and other foreign material, which necessitate redistillation of the water in an all-pyrex apparatus. The use of a Bunsen burner or an electric plate, as a source of heat, requires constant attention. The occasional cracking of the distillation flask when allowed to run dry, or after prolonged use, is a source of expense. Electrical heating elements can be employed; however, when in constant use, replacements are required.

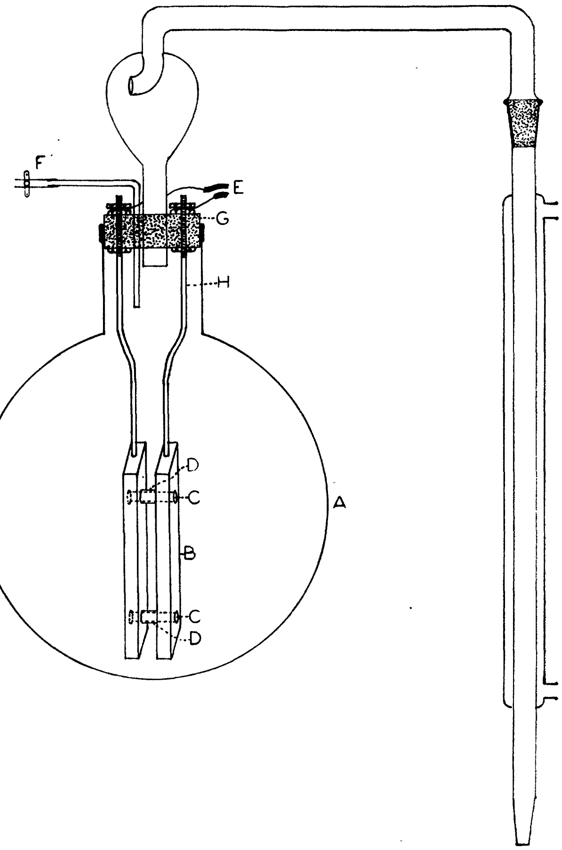

Fig. 1. (A) 5-liter flask. (B) Carbon electrodes, approximately 6 × 40 × 140 mm, which can be obtained at any electric motor shop. (C) Glass rods, 5 mm in diameter, with flattened ends to hold the carbon electrodes in place. (D) Glass tubes, 7 mm long, fitting snugly over the glass rods to keep the carbon electrodes apart. (E) Electric terminals connected to a 110-volt A.C. current. (F) Screw clamp on the water inlet from a 5-gal reservoir, adjusted so that the rate of the water dripping into the flask is in equilibrium with the rate of distillation. (G) Rubber stopper. (H) Brass rods, approximately 5 mm in diameter, fitted into the carbon electrodes and threaded on the upper end. The rods pass through the rubber stopper and are held in place with burrs.

It appears that a still (Fig. 1) which is easily constructed and requires a minimum amount of care and expense for its maintenance may well be of interest to other workers. It has the added advantage that the electric current to the heating element is automatically broken when the level of the liquid in the flask falls below the electrodes.

To operate the still, approximately 100 mg of sodium chloride are placed in the flask (*A*) to serve as a partial conductor for the current. The flask is filled about half full of water and the distilled water from a reservoir is allowed to drip into the flask at the same rate as the water is distilled. When this equilibrium is attained,