reagents. Following paper chromatography as little as 1 $\mu$g $\beta$-PEBG has been detected with these reagents. The detection limitations of smaller amounts of $\beta$-PEBG following chromatography have not been investigated.

No differences have been noted between the color tests given by $\beta$-PEBG free base and the salts mentioned. The alkali in these color tests would be expected to convert these salts to the free base[2] by proton removal. Therefore, it appears likely the free base is produced as an intermediary in reactions involving the salts investigated.

These location reagents have been reported to be useful for distinguishing structurally dissimilar guanidines, and to give positive color reactions with certain monosubstituted guanidinium compounds, but not with N,N'-disubstituted guanidines[1]. $\beta$-PEBG can be represented structurally as a monosubstituted or an N,N'-disubstituted guanidine. Since $\beta$-PEBG gives a positive test with all three reagents, the data have been interpreted as indicating $\beta$-PEBG behaves as a monosubstituted guanidine in these color tests. None of the colorimetric reactions mentioned is specific for $\beta$-PEBG, and detection requires prior purification of $\beta$-PEBG from other guanidinium compounds.

*Acknowledgements*

The authors are indebted to Dr. HARVEY S. SADOW, U.S. Vitamin and Pharmaceutical Co. who kindly furnished samples of $\beta$-PEBG and a grant-in-aid. This work was also supported in part by a Training Grant No. T1 AM 5230, National Institute of Arthritis and Metabolic Disease, USPHS.

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Received May 29th, 1964

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## A new technique for preparative paper chromatography

Preparative partition-chromatography techniques have been reviewed in several recent treatises on chromatography[1–3]. Many techniques that depend upon increasing the mass of the stationary phase are cumbersome and tedious or have low capacity[4–10]. The continuous methods[11,12], which require elaborate, synchronous mechanical equipment, are not used extensively although commercial equipment has been available.

The technique outlined in this report requires only readily available laboratory supplies and equipment. It has high, adjustable capacity and resolving power, simplicity, convenience, and versatility that facilitates multi-directional fractionations and the use of glass fiber, cellulose acetate, ion-exchange or any partition media available in tape form.

Fig. 1 shows a diagrammatic cross-section of the system. It is composed of a roll of commercial filter paper tape (A) from which a central portion has been unwound to accommodate a segment of the same filter paper tape (B) which is impregnated with a

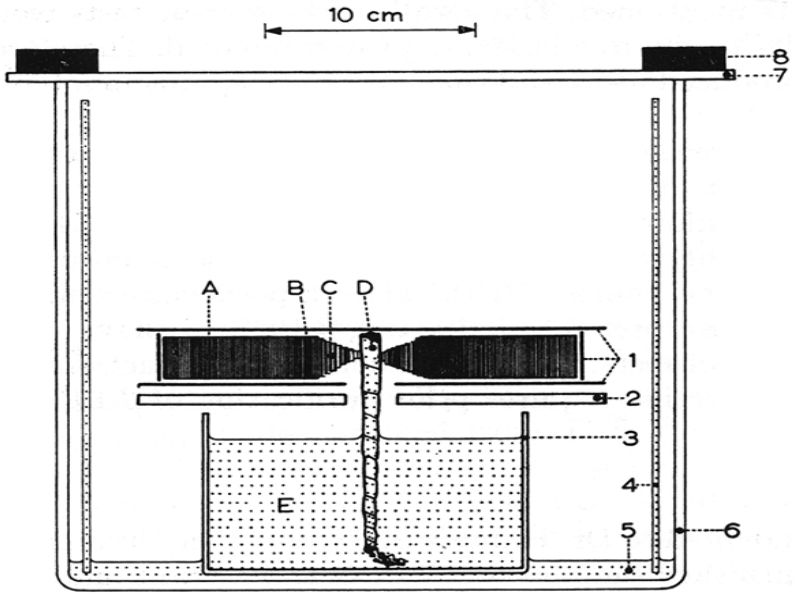


Fig. 1. Diagram of cross-section of assembly for preparative paper chromatography. A = filter-paper-tape roll, B = origin containing mixed solutes; C = distributor; D = wick; E = solvent reservoir; 1 = fluorocarbon-plastic film; 2 = glass plate; 3 = pyrex dish; 4 = blotting paper; 5 = solvent for chamber saturation; 6 = pyrex jar; 7 = transparent plastic cover; 8 = weight.

solution of mixed solutes and dried. The impregnated tape serves as the origin and is irrigated by a distributor (C) wound from progressively wider filter paper strips to which solvent is fed by a cylindrical filter paper tape wick (D) containing a core of cotton fibers. The wick dips into the irrigating solvent (E). All surfaces except the wick are covered with non-wettable plastic film. A glass plate with a hole in the middle to accommodate the wick supports the tape roll over a dish of irrigating solvent. The assembly is housed in a covered glass jar containing a small amount of solvent and lined with blotter paper to minimize solvent evaporation from the chromatographic column. The origin is marked conveniently by rubbing a pencil lead along both edges of the dried solute-impregnated tape segment. The chromatogram is developed by radial, horizontal migration of solvent and solutes through and between adjacent layers of tape. If necessary, irrigation may be interrupted briefly to measure solvent or solute boundaries. Non-uniform radial or lateral solvent flow to top or bottom surfaces can be adjusted by changing the distributor configuration by gentle pressure or by manipulating the wick. Loci of colorless solutes can be located at any time by removing the plastic cover temporarily, pressing a disc of filter paper on the horizontal surface of the roll with the aid of a soft rubber roller, and staining the imprint with the reagents commonly employed for paper chromatograms. Imprints upon glass-fiber paper can be stained with corrosive reagents. Colorless solutes can also be located directly on the horizontal surfaces by applying small amounts of test reagents from a toothpick or micro-pipet, or by placing a thin strip of filter paper impregnated with reagent between the origin and solvent boundary. Normally, direct staining does not

interfere with further chromatographic development because only a small surface is involved. A radioactivity detector can be employed to locate radioactive zones on the original chromatogram or on imprints. A radiochromatogram strip-scanner can continuously inspect paper tape when the completed chromatogram is unwound and dried.

When the solvent has migrated to within a few millimeters of the edge of the roll, the wick is removed and final imprints are obtained. The distributor is replaced with a one-hole rubber stopper containing an axial rod that is used as an arbor for unwinding the roll. Imprints or surface markings serve as guides when the tape is unwound and segmented to recover resolved substances. Each segment can be extracted for the isolation of individual constituents or rechromatographed directly on a new assembly.

The rate of solvent migration depends upon the size and shape of the wick and distributor, the width of the paper tape, and the solvent characteristics. The capacity and resolving power of the column increases with increasing width of the tape and radius of the roll. A 4-cm × 200-meter roll of Whatman No. 1 chromatography paper* having a radius of 10 cm and a mass of 700 g was employed to separate 1.5 g each of L-proline and L-leucine.

A typical separation of a 3-component mixture containing 600 mg of amino acids illustrates the utility of the technique. The distributor was prepared from a standard 50 × 50-cm sheet of Whatman No. 1 filter paper. Strips 50 cm long and varying in widths from 0.5 to 2.5 cm were wound progressively around a 0.25-in. wood dowel so that the vertical thickness of the coil increased regularly with increasing radius as shown in Figs. 1 and 2. The distributor radius was 2.6 cm when the vertical thickness reached 2.5 cm. A mixture of 500 mg each of DL-valine, L-proline and L-arginine (as the monohydrochloride) was dissolved in 10 ml of water. The solution was transferred to a large watch glass and absorbed completely into two strips of 2.5-cm wide, Whatman No. 1 chromatography paper tape having a total length of 208 cm. The air-dried paper strips contained 7.2 mg of amino acid mixture per centimeter of tape length. An 83.5-cm strip of this tape containing 200 mg of each amino acid was marked along both edges to establish the position and width of the "origin." The strip was wound around the distributor, to extend the latter's radius by 1.3 mm. The central part of a 2.5-cm thick × 10-cm radius (370 g) roll of Whatman No. 1 chromatography paper was unwound to provide a 5.5-cm I.D. opening. With the aid of a few additional tape windings, the distributor-origin assembly was fitted snugly into the opening. Care was taken to align the helical winding with that of the roll. Slight counter-rotation of the distributor assembly helped to get an intimate fit between the two parts and subsequently a smooth, uniform solvent flow. A 1.1 × 10-cm, slightly conical, cylindrical wick was formed by pushing out the central section of a 10-turn coil of the same paper tape. The wick was mounted in the central opening of the distributor, with the apex of the truncated cone hanging about 7.5 cm below the lower horizontal surface of the roll. Absorbent cotton was stuffed into the hollow wick to increase solvent flow to the distributor. A 2.5-cm wide strip of fluorocarbon plastic tape was wound around the edge of the assembly to keep the tape from unrolling and to reduce evaporation from the solvent front. A thin sheet of the same plastic film was placed over the upper surface, and a similar sheet with a 2.5-cm central perforation was placed under the lower surface. An 0.5 × 23-cm glass plate with a 2.5-cm diameter hole in the middle sup-

* Reference to a company or product does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

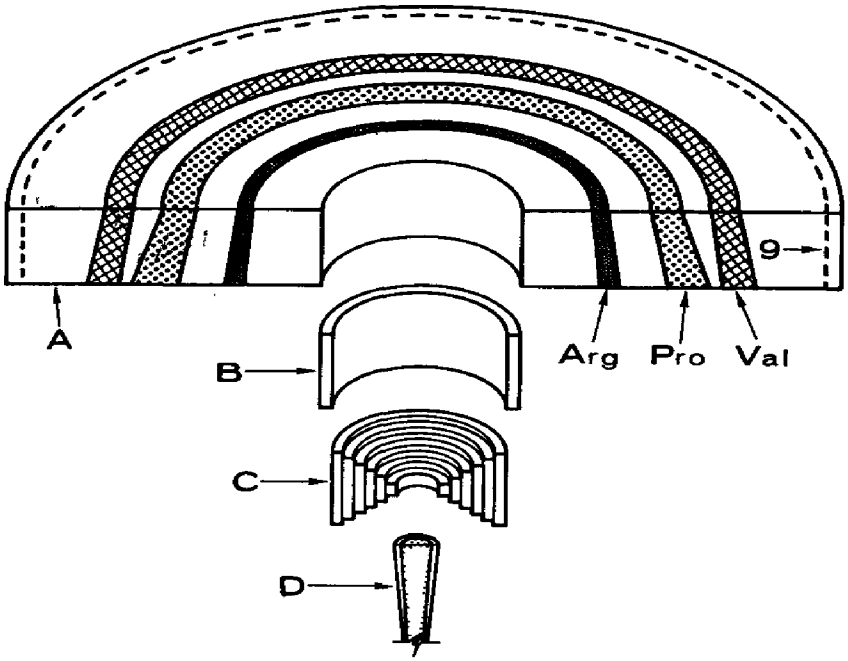


Fig. 2. Cutaway isometric projection of tape-roll chromatogram, based on imprints prepared from upper and lower surfaces. Symbols A, B, C, D are the same as in Fig. 1; 9 = solvent boundary.

ported the assembly over a pyrex dish containing about 1.5 l of irrigating solvent. The solvent mixture of *tert.*-butanol–85 % formic acid–water in the ratio 69.5:4.0:26.5 migrates slowly but separates amino acid mixtures effectively[13]. The entire assembly was housed in a 30 × 30-cm cylindrical pyrex jar, which was lined with solvent-impregnated blotting paper and sealed with a transparent plastic plate undercoated with silicone grease. At 21° the solvent moved 7.0 cm beyond the origin in five days. The wick was removed and imprints of the upper and lower surfaces were prepared on 24-cm filter paper discs. The imprints were immersed for a few seconds in a modified[13] ninhydrin dip reagent[14]. Intense colors appeared within 20 min at ambient temperature. Patterns on the two imprints were compared to estimate the distribution of the amino acids within the roll (Fig. 2). The tape was unwound on a lattice-rod reel and cut into three sections corresponding to the zones of the three amino acids. Each segment was wound into a separate coil, formed into a cone by extending the center section, and placed on a 9.0-cm I.D. Buchner funnel. The cones were extracted over a 24-hour period by dropwise addition of about 1 l of water from a separatory funnel. Each eluate was evaporated to dryness under reduced pressure. Yields of crude products were approximately 80–90 %. The valine contained a little proline because the irrigating solvent had migrated slightly faster on the lower part of the roll (Fig. 2). Arginine and proline were chromatographically pure although slightly yellow. Extended soaking of the paper tape cones did not remove all of the amino acids, especially if the tapes had dried thoroughly before extraction. Ninhydrin reacted on tapes that had been extracted for several hours in a Soxhlet extractor, indicating residual amino acids or partial interaction with the filter paper.

Some modifications of the basic procedure have been tried. The chromatographic process can be accelerated by reversing the wick so that it protrudes above the surface of the roll and irrigating it by an automatic, metered, solvent feed device mounted

above the assembly. However, separations are less satisfactory when the column is loaded heavily and equilibrium is not attained between the partitioning phases.

A mixture of dyes was separated by use of two types of two-directional procedures. After preliminary fractionation on a tape roll, a section of tape containing unresolved dyes was placed between a new distributor and tape roll and rechromatographed directly with a second solvent system to complete the separation. The second technique involved sectioning, drying, and rewinding of the unresolved zone into a tight coil. It was mounted between identical coils of fresh tape having the same diameter as the rewound segment. The assembly was placed in a dish of a second solvent that covered about two-thirds of the lower coil. Weights placed upon the upper-most coil improved surface contact. The assembly was covered by a glass jar. Ascending migration of solvent carried one of the unresolved dyes into the upper coil effecting two-directional, three-dimensional chromatography.

Eluting components from the periphery and collecting fractions in an automatic fraction collector might be expected to improve separations. The plastic strip around the edge of the column could be replaced with a filterpaper skirt having a serrated lower edge which would permit drop formation and collection in a funnel leading to a fraction collector. A similar arrangement with the skirt and wick immersed in separate buffer reservoirs containing electrodes attached to a power supply might provide a convenient system for preparative electrophoresis of mixtures that are not readily separated by partition chromatography.

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Received May 25th, 1964

* A laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.