A METHOD TO REDUCE SOLVENT FLOW IN PAPER CHROMATOGRAPHY

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A special geometric shape has been designed to effect a decrease in the rate of solvent movement on paper. Such slow running papers have been used with advantage in two dimensional paper chromatography of amino acids. These have also been used to study the effect of rate of solvent flow on Rf values of a few amino acids.

The diffusion of spots in paper chromatography depends upon, the nature of the constituents, the nature of the paper and the rate of solvent movement on paper; with slow solvent movement the spots obtained are compact and separation is better. Interesting techniques have been described to control the rate of solvent movement on paper. Mueller (1950) attached a slow running paper at lower edge of a fast running paper through a double row of stitches by means of a sewing machine. Irreverre and Martin (1954) have described an ingenious technique in which solvent is fed to the paper with a wick of cotton strands, and the rate solvent flow can be controlled by increasing or decreasing the length of the wick or by varying the number of strands in the wick. Muller and Clegg (1951) and Kawkabany and Cassidy (1950), using filter paper strips of a variety of shapes, found that rate of flow is affected by the geometric shape of the paper. We were primily interested in increasing the compactness of spots and to start with, we tried differently directed diffusion currents of the solvent near the mixture spot. The study led us to a simple geometric shape of paper which could reduce solvent movement and the size of the spots effectively. These papers were called as the wick papers (Fig. 1).

METHOD

Preparation of the wick paper.—Following is a general plan, described with reference to 2/8 and 4/8 wick papers, which was followed for cutting different wick papers.

Preparation of the base wick.—Three parallel lines AB, CD and EF were drawn at distances of 2 cm., 4.4 cm. and 5.8 cm. respectively from the lower

edge of the paper (Fig. 1). On the line AB two points G and H were taken so that GH represents middle half for 4/8 wick paper or middle one fourth for 2/8 wick paper. The point G was joined with E and the point H with F. Line K G L and I H J were drawn through the point G and H respectively, perpendicular to the line AB and cutting other lines as shown. The base wick G H J L was formed when the paper was cut along the lines J H F and L G E.

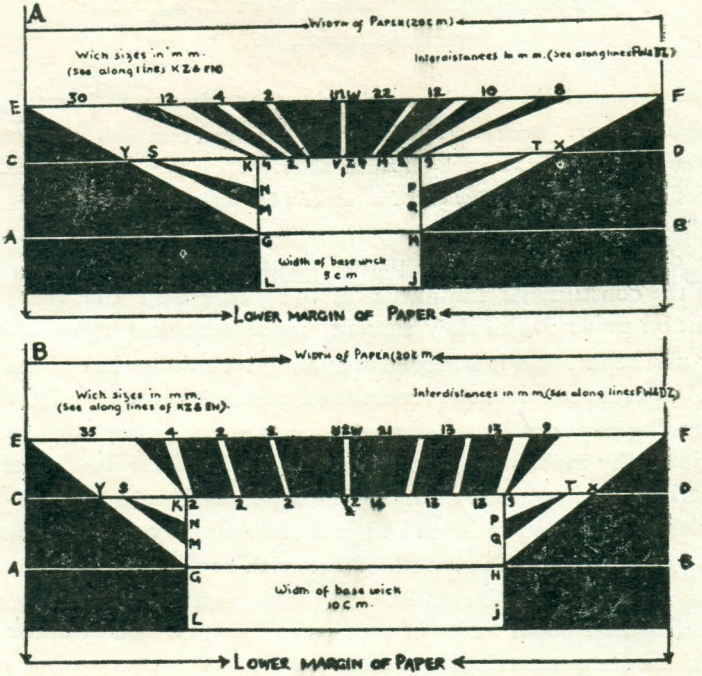


Fig. 1. Slow Running Wick Papers.

A. 2/8 wick paper (2/8 is the ratio of the width of the base wick to that of the paper.)

B. 4/8 wick paper.

Dark portions represent the areas of the paper which have been removed. (Also in Fig. 2)

Preparation of side wicks.—N. M, which is equal to P Q, represents middle one fourth of the distance K G or I H. Points T and S were marked on the line C D, so that XT=YS=MG or HQ. Paper enclosed within triangles, M N S and P Q T, was removed. K N, M G, H Q and I P were the lower ends of the two connecting wicks on sides.

Preparation of the connecting wicks.—The principle underlying the preparation of the connecting wick (i.e. the wicks connecting the base wick with the

rest of the paper) was that sizes of the wicks go on decreasing from without inwards, and their interdistances go on increasing. There is no hard and fast rule in selecting the wick sizes and the interdistances, and any combination will do, provided, when the base wick is dipped in a solvent, an almost horizontal solvent front is attained within a distance of 1.5 cm. from above the tops of the wicks (Fig. 1). However a slight change on this or that side does not matter. In actual practice the various lines and points described above were drawn on a thick paper and holes were made through the various points L, J. G, H, M, N, P, Q, K, S, I, T, E, F, U, W, V, Z, etc. This paper was used as a stencil to mark different points through the pin holes on another paper which was to be cut into a wick paper. These points were then joined suitably and the required areas of paper removed with a sharp blade. Preparation of a wick paper in this manner took about 8 to 10 min.

Solvents.—

Various new solvents were studied and used with advantage. But only the two employed in the study of wick papers in two dimensional paper chromatographic technique are mentioned here.

The first solvent consisted of a mixture of water saturated phenol, isopropyl alcohol and isoamyl alcohol (14 : 4 : 2 v/v). A petri dish containing a little liquor ammonia and another containing a few ml of 2 percent sodium cyanide solution were placed inside the chamber in which development was undertaken.

The second solvent consisted of a mixture of isopropyl alcohol, n-butyl alcohol, isoamyl alcohol, formic acid and water (5 : 3 : 2 : 1.5 : 2 v/v).

Procedure.—

Studies with these wick papers (cut from sheets of filter paper Whatman No. 1) were made with ascending technique of paper chromatography, and papers were supported in a vertical position by clipping their upper ends to the threads fixed inside the chromatographic cabinets. The lower ends of the papers were fixed inside the elongated solvent throughs with the help of supporting rods. Other details were similar to those of conventional procedures.

RESULTS AND DISCUSSION

We could not achieve compactness in spots by the use of differently directed flow currents under the mixture spot. Fig. 2, illustrates the effect of the variously directed solvent currents on the size and shape of the spots obtained. The spots were compact in cutting E because of slow solvent

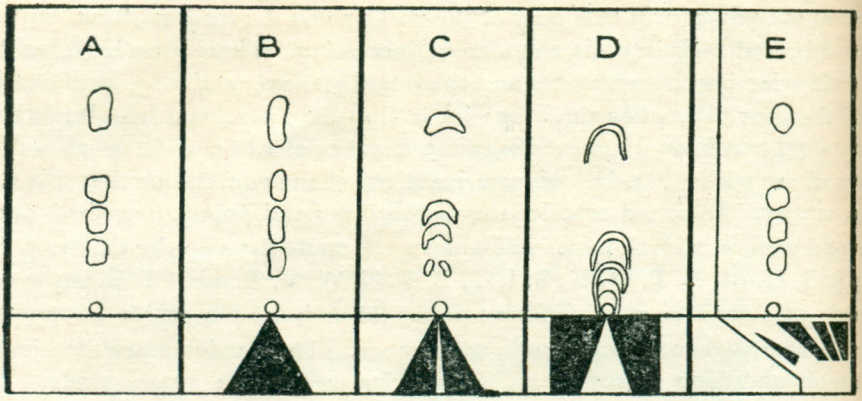


Fig. 2

movement. This cutting was later developed into wick papers detailed already. However some other methods were also tried to slow down the flow rate. One of them consisted in dividing the lower edge of paper by equidistant parallel cuts into a large number of similar paper strips and removing the alternate ones. In another attempt, instead of removing a part of the paper as described above, its capillaries were blocked with the help of molten paraffin wax. But none of these methods proved satisfactory.

Rate of solvent flow depends upon the ratio of width of the base wick to that of the paper (Fig. 3). Different ratios between 7/8 and 1/8 were studied, but no attempt was made to go beyond a ratio of 1/8, since in such cases proper sizing of the different connecting wicks becomes difficult.

In two dimensional paper chromatography, the wick papers may be used with advantage for the first development. For the second also, the wick system may be used but with due care that sizing of the paper is such as to give a horizontal solvent front ; otherwise it may not be used at all.

The effect of rate of solvent movement on the spot size is illustrated in Fig 4. It may be noted that the effect is more pronounced in amino acids with higher Rf values and that the vertical diameter is affected much more than the transverse one.

In Fig. 5, three retraced chromatograms are shown for comparison. All the papers were subjected to an indentical second running of 8 hrs (and without wicks). The control paper A and the 2/8 wick slow running paper B were removed after 12 hrs in the first development. On comparing the two chromatograms A and B, it is evident that in the paper B, although the spots were more compact, the separation was not much improved.

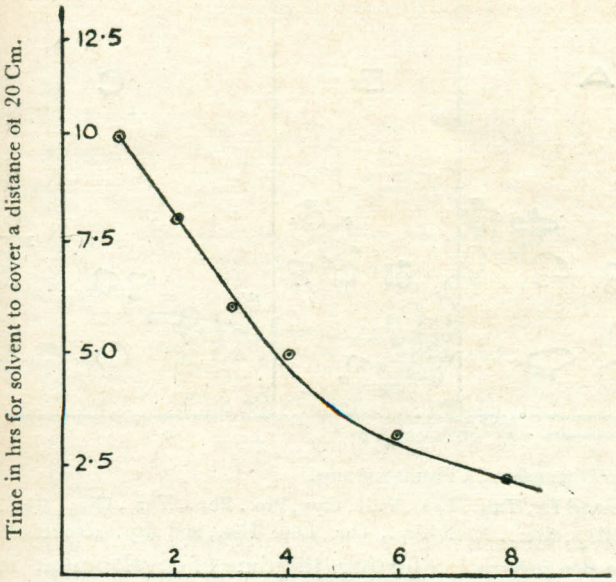


Fig. 3 $\frac{\text{width of base wick}}{\text{width of paper}} \times 8$

	A	B	C	D
Methionine Rf. 0·57	16/11	15/10	12/10	11/10
Tryptophane Rf. 0·56	20/8	17/7	15/7	14/7
Valine Rf. 0·52	11/10	10/10	10/10	9/10
Glycine Rf. 0·12	12·5/11	12/11	12/11	12/10·5

Fig. 4. Effect of solvent flow Rate on spot size.

Under A, B, C and D are shown spots from the control paper and 4/8, 2/8 and 1/8 wickpapers, respectively, under each spot is given its length/width in mm.

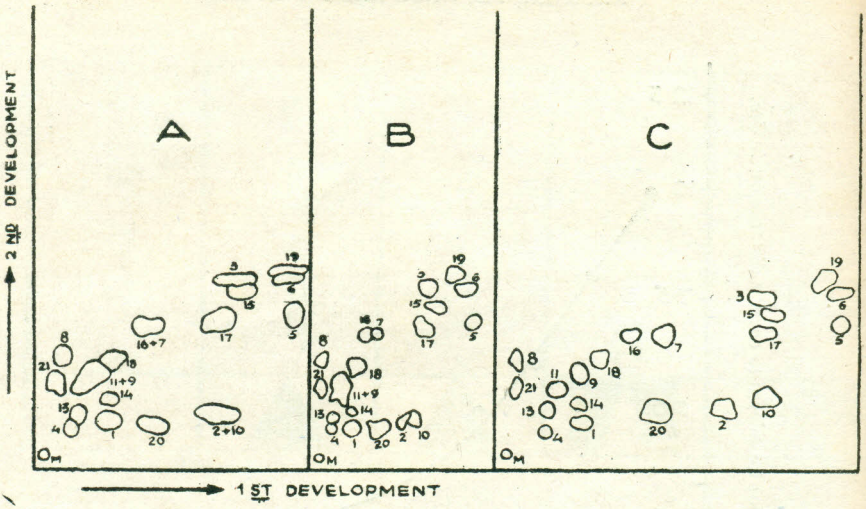


Fig. 5. Two Dimensional Chromatograms.

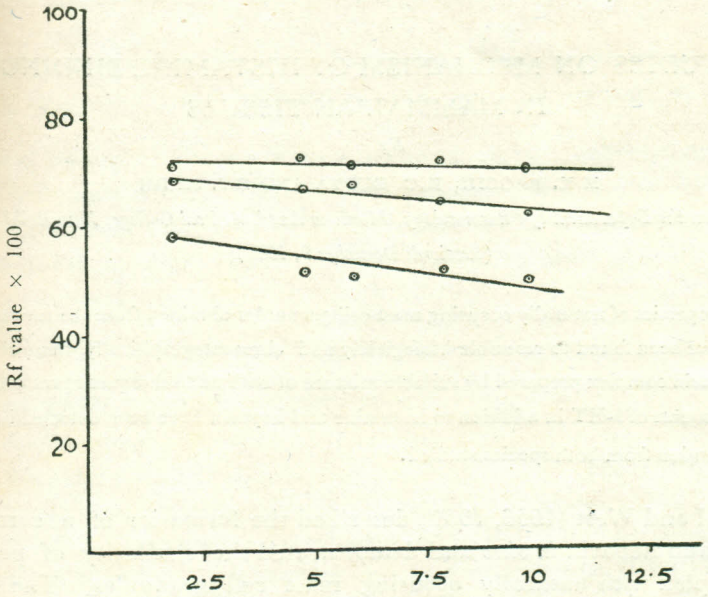
Nos. 1 to 21 stand for, Tau., Lys., Val., Gys , Pro , Phe., Tyr., Glu., Gly., Arg., Ser., Asp-NH_2., Glu-NH_2., Met., Ala., Try., Thr., Leu., His., and Asp. respectively.

To improve the separation further, the time of development in the first running was increased from 12 hrs to 36 hrs as shown in chromatogram C. The control paper simultaneously developed with C (not shown in the Figure), however, depicted only diffuse bands instead of spots.

In two dimensional paper chromatography of amino acids, when aqueous phenol is used as first solvent and development is undertaken in the presence of a little ammonia, the chromatograms are not suitable for quantitative estimations (Block, Durrum and Zweig, 1958), This is because the spots obtained with the first solvent under these conditions are quite large and elongated. With the wick papers, however, the phenol solvent used in our study did not suffer from this drawback.

The effect of rate of solvent flow on Rf values of amino acids is a controversial issue. Kawkabany and Cassidy (1952), using filter paper strips and ascending techniqne, showed that under their experimental conditions, Rf values of valine and glycine did not change by flow rates. However, using different filter papers (Kowkabany and Cassidy, 1950), it was observed that faster ones gave higher R_f values. On the other hand, Irreverre and Martin (1954) observed that Rf values are related to flow rates and this has been confirmed by us (Flg. 6).

Although all these observations with the wick papers have been made with ascending technique of paper chromatography, these papers may be



Time in hrs for solvent to Cover a distance of 20 Cm.

Fig. 6

more suitable for descending technique, where rate of deovlopment is not decreased with advance of solvent front. In the ascending technique, this benefit of separation, with slow solvent movement, may be obtained simply by spotting the mixture sufficiently above the two continuous edges of the paper (Saini, Singh and Singh, 1963).

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