## ASCENDING (PAPER) CHROMATOGRAPHY OF SUGARS BY THE MULTIPLE DEVELOPMENT TECHNIQUE

JEANES *et al.*[1] HAVE DESCRIBED A MULTIPLE development technique for the ascending (paper) chromatography of sugars. They used two-component sugar mixtures and were able to derive equations on a theoretical basis from which the number of runs necessary for the maximum distance of separation of two sugar spots could be calculated.

In the present communication, results obtained by extending the technique of Jeanes *et al.* to the resolution of a mixture containing five sugars (lactose, maltose, dextrose, galactose and arabinose; each sugar at 1 per cent level) are given. The procedure adopted was essentially the same, but rectangular strips of filter paper (38×4 cm., Whatman No. 1) were used instead of cylindrical sheets; and the apparatus[2] was considerably simpler, being easily assembled from a specimen jar, a beaker and a few bits of glass tubing, plasticine and wire clip. Certain difficulties inherent in the handling of large wet filter paper chromatograms could thus be avoided and the solvent loss was also minimized. The solvent system used was a mixture of n-butanol, ethanol

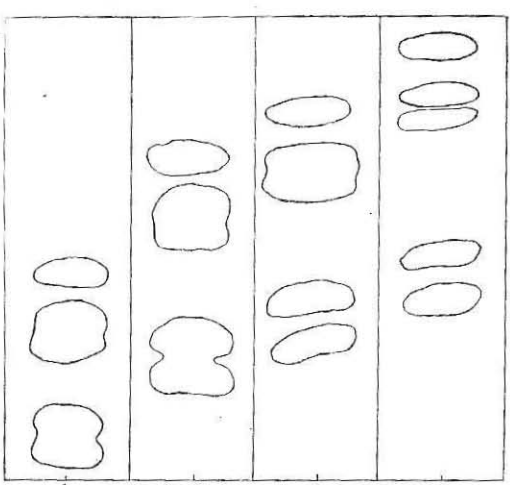


FIG. 1 — PROGRESS OF SEPARATION FROM A MIXTURE OF FIVE SUGARS FOR DIFFERENT RUNS OF THE SOLVENT [A, 2 runs; B, 3 runs; C, 4 runs; and D, 5 runs]

TABLE 1 — DISTANCE OF SEPARATION BETWEEN SUGAR SPOTS ( CM.)

| Sugar mixture | No. of runs* | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Lactose-maltose | 0·62 | 0·80 | 1·10 | 1·20 | 1·40 | 1·50 | 1·50 |
| Lactose-dextrose | 1·74 | 3·30 | 4·40 | 5·10 | 5·40 | 5·50 | 5·30 |
| Lactose-galactose | 1·90 | 4·00 | 5·20 | 6·20 | 6·50 | 6·70 | 6·40 |
| Lactose-arabinose | 3·40 | 5·30 | 7·10 | 7·80 | 8·10 | 8·00 | 7·00 |
| Maltose-dextrose | 1·12 | 2·50 | 3·30 | 3·90 | 4·00 | 4·00 | 3·80 |
| Maltose-galactose | 1·28 | 3·20 | 4·10 | 5·00 | 5·10 | 5·20 | 4·90 |
| Maltose-arabinose | 2·78 | 4·50 | 6·00 | 6·60 | 6·70 | 6·50 | 6·20 |
| Dextrose-galactose | 0·16 | 0·70 | 0·80 | 1·00 | 1·10 | 1·20 | 1·10 |
| Dextrose-arabinose | 1·66 | 2·00 | 2·70 | 2·70 | 2·70 | 2·50 | 2·40 |
| Galactose-arabinose | 1·50 | 1·30 | 1·90 | 1·60 | 1·30 | 1·30 | 1·30 |

*Total distance travelled by the solvent = n×26 cm., where n is the number of runs.

TABLE 2 — NUMBER OF RUNS REQUIRED FOR MAXIMUM DISTANCE OF SEPARATION OF SUGAR SPOTS

| | Calculated values | | | | | Observed values | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | L | M | D | G | A | L | M | D | G | A |
| Lactose (L) | — | 7 | 6 | 6 | 5 | — | 6 | 6 | 6 | 5 |
| Maltose (M) | 7 | — | 6 | 5 | 5 | 6 | — | 5 | 6 | 5 |
| Dextrose (D) | 6 | 6 | — | 4 | 3 | 6 | 5 | — | 6 | 4 |
| Galactose (G) | 6 | 5 | 4 | — | 3 | 6 | 6 | 6 | — | 3 |
| Arabinose (A) | 5 | 5 | 3 | 3 | — | 5 | 5 | 4 | 3 | — |

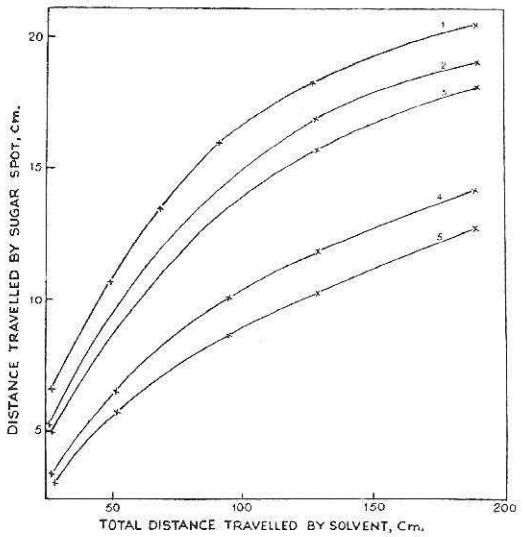


FIG. 2 — DISTANCE TRAVELLED BY INDIVIDUAL SUGAR SPOTS *vs.* TOTAL DISTANCE TRAVELLED BY SOLVENT [1, arabinose; 2, galactose; 3, dextrose; 4, maltose; 5, lactose]

and water[3], and the spray reagent was made up of benzidine and trichloracetic acid as recommended by Bacon and Edelmann[4].

The progress of separation of the sugars from the mixture is indicated in Fig. 1. In Fig. 2 the distances travelled by the individual sugar spots have been plotted against the distances travelled by the solvent. For determining the number of runs ( $n_{max.}$) in which the distance between the centres of any two sugar spots is a maximum, the respective distances were read off from Fig. 2 for a constant distance ( 26 cm.) travelled by the solvent per run ( TABLE 1 ). These observed values of $n_{max.}$ have been given in Table 2 together with the theoretical values calculated from the formula

$$n_{max.} = \frac{\log R_{f_1} - \log R_{f_2}}{\log (1-R_{f_2}) - \log (1-R_{f_1})}$$

given by Jeanes *et al.*

It is seen from Table 2 that there is good agreement between the observed and calculated values. This agreement is of added interest since hitherto values of $n_{max.}$ do not appear to have been determined for mixtures containing as many as five sugars. The enhanced utility of the multiple development technique, especially in quantitative analysis, is also evident.

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1. JEANES, A., WISE, C. S. & DIMLER, R. T., *Analyt. Chem.*, **23** ( 1951 ), 415.
2. VISWANATHAN, R., *Proc. 40th Indian Sci. Cong. Ass.*, 1953, Pt. III, 281.
3. BRYSON, J. L. & MITCHELL, T. J., *Nature*, **167** ( 1950 ), 864.
4. BACON, J. S. D. & EDELMANN, J., *Biochem. J.*, **48** ( 1951 ), 115.