

Paper chromatographic front indicators

On carrying out paper chromatography with sheets of paper it is found that solvent fronts are never straight.

MÜNZ¹ explained in detail why solvent fronts run in an uneven manner on sheets of smooth filter paper rolled up into a cylinder. In most cases conditions of steady run cannot be maintained, and this results in uneven solvent fronts which are rather difficult to mark. Similarly, difficulties are encountered when attempting to mark solvent fronts in the case of volatile solvents. To facilitate the technique of paper chromatography it therefore seems necessary to evolve a method for marking front lines.

In the present experiments, the behaviour of 31 dyes in different solvents was examined. The dyes tested consisted of various types (phenazine, azo, triphenylmethane, indigoid, xanthene, thiazine, phthaleine) that are often applied in most laboratories. Most of the dyes were used in aqueous solution; those that are insoluble in water were dissolved in 96% ethanol. Of the usual solvents employed in chromatography, only twelve that are most often applied, were selected. Experiments were carried out with Whatman No. 1 filter paper, but sometimes also replicate determinations were made with Schleicher and Schüll 2043 b type filter paper, to find out whether there are differences between the two papers when they are tested with the same dyes in the same solvent.

When the ascending technique was applied the dyes were transferred to paper sheets of 25 × 21 cm, on a "starting line" 2.5 cm from the top of the paper. Lines 1 cm long were drawn with each dye by means of a thin glass capillary, leaving free spaces of 0.25 cm between consecutive lines. After rolling up the sheets, chromatograms were run in two large beakers with ground edges, one of which was inverted and placed on top of the other. The beakers were closed airtight by applying a vaseline film to their ground edges. Chromatograms were run in a thermostat at 23–25°.

When using the descending technique, sheets of 25 × 25 cm of smooth filter paper were employed, and the starting line of the dyes was located 2.5 cm from the bottom of the sheet.

The dye concentrations varied between 0.01 and 1.0%, according to the intensity of the colour.

It is extremely important that the dye solution, on leaving the thin capillary, should form a stripe not wider than 1–3 mm when the front line is being marked with a ruler.

Running was stopped when the solvent front had passed 1.0 to 1.5 cm beyond the starting line of the dyes. After removing the solvent, the front line should be marked by pencil, because the dye line may become diffuse (indistinct) or may migrate during spraying with the developing reagent or, in two-dimensional chromatography, when a second solvent is applied.

In practice, it proved advantageous to draw the dye line 0.5–1.0 cm before the place where the solvent front was expected to arrive. In the case of a rather straight

TABLE I

Dye	Whatman No. 1													S & S 2043 b			
	Solvent													Solvent			
	a	b	c	d	e	f	g	h	i	k	l	m	n	c	d		
Safranine TH									++							+	
Baumwollrot 4 BC				+													++
Bismarck Brown							++										++
Crystal Violet	++	+	+		++	++		++									++
Erioglaucine A					+												
Auramine O					++												
Orange II					++												
Rhodamine GGH	++	++	++	+	++	++	++	++									++
Metanil Yellow					++												++
Methylene Blue																	
Direct Green B						++											
Solarflavine 5 G						+											
Rhodamine ZS	+	+	+		++	++											+
Erythrosin	++	++	++	+	++	++											++
Indigocarmine																	
Rosaniline	++	++	++	+	++	++	+	++	++	++	++	++	++	++	++	++	++
Nigrosine, ethanol-soluble																	
Nigrosine, water-soluble																	
Bromocresol Green					++	++											
Bromocresol Red					++	++											
Fuchsin	+	+	+		++	++											++
Malachite Green	+	+	+	++	++	++											++
Methyl Blue																	
Eosin-Na	++	++	++	+	++	++											++
Fluorescein-Na	++	+	+	+	++	++											++
Sudan III	++	++	++	++													++
Litmus																	
Lacmoid																	
Methyl Red	+	+	++	+													+
Methyl Green																	
Direct Turkish Blue																	

++ = extremely sharp front line, + = less sharp front line.

solvent front, even less suitable dyes yield sharp lines after migrations of 0.5 cm. The 0.5–1.0 cm run of the dyes corresponds even in short-distance runs (20 cm) to the R_F interval of 0.95–1.00, so that there is little risk of the dyes causing interferences, since only a few substances with R_F values exceeding 0.95 are known.

The behaviour of the dyes in various solvents is summarized in Table I.

The solvents applied were the following:

- (a) Butanol–acetic acid–water (4:1:1).
- (b) Butanol–acetic acid–water (4:1:5). In solvent mixtures (a) and (b) suitable dyes partly remained at the site of application, Sudan III and Methyl Red giving the smallest residual spots.
- (c) Ethyl acetate–acetic acid–water (3:1:1). Erythrosin and Sudan III completely disappeared from the starting line.
- (d) Tetrahydrofuran–water (3:2). Malachite Green, Sudan III and Methyl Red gave no spots at the starting line.
- (e) Acetone–water (3:2). Sharp front lines without residual spots were given by Auramine O, Orange II, Rhodamine GGH, Metanil Yellow, Erythrosin, Eosin-Na, Fluorescein-Na and Bromocresol Red. The other suitable dyes left small residual spots at the starting line.
- (f) Pyridine–water (13:7). Auramine O, Metanil Yellow, Rhodamine ZS, Bromocresol Green, Bromocresol Red, water-soluble Nigrosine, Malachite Green and Lacmoid proved to run without leaving residual spots.
- (g) Butanol–ethanol–water (5:1:4). All the suitable dyes left residual spots at the starting line.
- (h) Collidine, saturated with water. Only Sudan III proved to run without leaving a residual spot, other dyes gave small spots.
- (i) Phenol, saturated with water. Of the suitable dyes, Bismarck Brown, Rhodamine ZS, Fuchsin and Malachite Green gave very small spots.
- (k) Amyl alcohol, saturated with water. All suitable dyes gave appreciable spots at the starting line. In this case the solvent front should not pass more than 1 cm beyond the starting line.
- (l) Butanol–3% ammonia. All dyes yielding sharp front lines left appreciable residual spots at the starting line. The dyes behaved as if they were composed of two ingredients, one of them remaining at the starting line, the other migrating with the solvent front.
- (m) Butanol–formic acid–water (77:10:13). Only Erythrosin proved to run without leaving a residual spot, all the other dyes gave appreciable residual spots at the starting line.

It was our general experience that with all solvents tested the yellow dyes (Auramine O, Metanil Yellow, Solarflavine 5 G, Fluorescein-Na) gave sharp lines of satisfactory strength only in high concentrations. In the case of lower dye concentrations, the solvent front can be marked by transillumination.

The migration of the dyes with the solvent front was practically the same in solvent systems of identical components but of various concentrations.

Our experiments proved that, on using Whatman No. 1 filter paper with both the ascending and the descending technique, certain dyes (such as Crystal Violet, Rhodamine GGH, Rhodamine ZS, ethanol-soluble Nigrosine, Malachite Green, Sudan III) are suitable for marking front lines of nearly all the solvents used in paper chromatography.

Plant Research Station, Mezöhegyes (Hungary)

ENDRE SOMFALVY
KLARA S. AUSCH

¹ T. MÜNZ, *Naturwissenschaften*, 41 (1954) 553.

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BOOK REVIEWS

Gas Chromatography (A Symposium held under the auspices of the Analysis Instrumentation Division of the Instrument Society of America, August 1957), edited by V. J. COATES, H. J. NOEBELS AND I. S. FAGERSON. Published by Academic Press Inc., New York, July 1958, 313 pp. Price \$ 10.00; after October 1st, 1958, \$ 12.00.

In a paper given at this Symposium, A. J. P. MARTIN pointed out: "the present position of gas chromatography... seems to be the rapid application of well-known methods to an infinity of problems", and the other 26 papers amply supported this statement. It is extremely valuable to hold formal and informal discussions between experts and beginners and also persons who may have only just realised the great potentialities of Gas Chromatography. This book provides a record of the papers presented and the formal discussions after each paper.

The papers can be divided into five main groups:

- (i) Considerations of the Theory of Gas Chromatography,
- (ii) The Application of Gas Chromatography Instrumentation in the Laboratory,
- (iii) The Analysis of High Boiling Materials by Gas Chromatography,
- (iv) The Application of Gas Chromatography to the Purification of Chemicals, and
- (v) Gas Chromatography Instrumentation for Continuous Automatic Analysis.

Three papers in particular may be mentioned for their probable influence in the future. The first by GOLAY is entitled "Theory and Practice of Gas-Liquid Partition Chromatography with Coated Capillaries". The technique described will probably become widely used for the achievement of very high resolution or very short analysis time, provided that a small sample and a sensitive detector are used. The second paper by PHILLIPS on "Gas Chromatography Instrumentation for the Laboratory" describes the use of metal salts, e.g. zinc stearate, as column liquids. Highly specific retardation of solutes can be achieved with these metal salts and this principle can be applied to many analytical problems. These studies should be of interest as a model for work at