

Semiquantitative evaluation of the spots of tar polycyclic hydrocarbons on paper chromatograms by measurement of their horizontal diameters

FISHER and coworkers¹ have described a simple but not preparative method for the quantitative evaluation of spots directly on paper chromatograms by measuring their area. When the spots are round such an evaluation can be made even more simply by marking their lateral borders (irrespective of how the spots are detected), and measuring the horizontal diameters with dividers. This method is applicable even when the spots are not completely separated from each other. This is the case, for example, with chromatograms of the neutral portion of tars, using the solvent system previously described², where the R_F values decrease with an increase of the molecular weight. Here the spots, which are visible in transmitted U.V. light, follow each other closely, their lower parts being overlapped by the upper parts of the following spots. This is due to the fact that these mixtures are very rich in compounds of similar molecular weight, some of which are even isomers.

In this solvent system the 3:4-benzopyrene spot is separated completely from 1:2-benzopyrene, 1:12-benzoperylene (the authentic compound had an R_F value of 0.22, and showed violet fluorescence), and very probably partially from perylene (R_F 0.31, weak blue fluorescence in the chromatogram of coal tar), when the spots are small enough. Fig. 1 shows the 3:4-benzopyrene spot with its nearest and

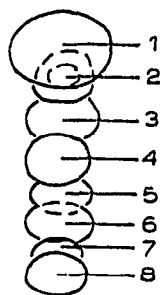


Fig. 1. R_F values, fluorescence, and identification of some spots on a coal tar chromatogram. (1) 0.38, emerald green, fluoranthene. (2) 0.36, green with violet ring, pyrene. (3) 0.35, blue-violet, chrysene. (4) 0.32, yellowish green, 1:2-benzopyrene. (5) 0.31, blue, very probably perylene. (6) 0.28, violet, 3:4-benzopyrene. (7) violet fluorescing zone belonging to unidentified compounds (possibly 1:12-benzoperylene, anthanthrene, and 1:2;5:6-dibenzanthracene). (8) 0.25, greenish yellow, unidentified (possibly one of the dibenzanthracenes).

farthest neighbours on the chromatogram of coal tar, when the spots are not small enough. Fig. 2 and Table I give an example of this method of calibration for the 3:4-benzopyrene spot. Proof of the separation of some hydrocarbons in this solvent system was given previously³, and the complete method for the determination of polycyclic hydrocarbons in air was described³. Such a calibration need not be restricted to chromatograms of tars, but it could be generalized and applied to other overcrowded chromatograms.

A glass cylinder with ground-in stopper (height 40 cm, diameter 20 cm) was

used as a chromatographic chamber, while spotting was carried out with a Linderstrøm-Lang automatic pipette of 1.1λ (twice half a pipette was spotted). The height of the level of the ascending mobile phase (methanol saturated with paraffin oil) was

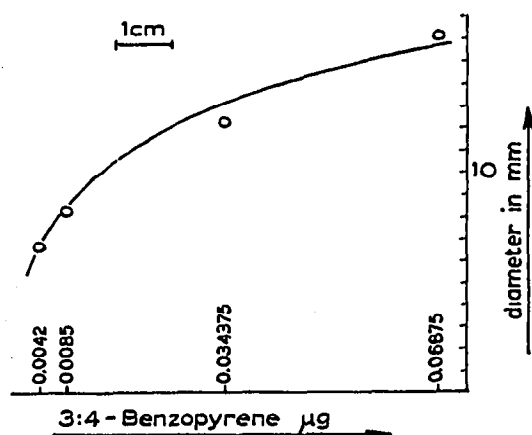


Fig. 2. Calibration curve of the spot of pure 3:4-benzopyrene.

1 cm from the bottom. The developing time was 6 h at room temperature. Standard solutions containing 1.0 mg of 3:4-benzopyrene per ml in a mixture of paraffin oil-chloroform or benzene (25:75) were always gradually diluted with this mixture until the concentration was half the original value. The starting line was 1.5 cm from

TABLE I

Quantity of 3:4-benzopyrene in μg spotted on the origin	Average diameters of the spots in mm
0.0021	0.0
0.0042	6.7
0.0085	8.3
0.0343	12.2
0.0687	16.0
0.1375	17.3
0.2750	19.2
0.4125	20.8
0.5500	21.4

the edge of the paper (0.5 cm above the level of the mobile phase). The demarcation line of distinguishable fluorescence (in comparison with the location of the lateral borders of the spot) was drawn. The impregnation was carried out by dipping the rolled sheet of Whatman No. 4 paper (38 cm high) into a glass cylinder, 6 cm in diameter and 40 cm high, containing the impregnation solution: 10% petrol ether in paraffin oil (medicinal quality). Coal tar solution without any previous adsorption chromatography was spotted.

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*Institute of Industrial Hygiene and Occupational Medicine,
Bratislava (Czechoslovakia)*

ERNEST MALÝ

¹ R. B. FISHER, D. S. PARSONS AND G. A. MORRISON, *Nature*, 161 (1948) 764.

² E. MALÝ, *Nature*, 181 (1958) 698.

³ E. MALÝ, *Pracovní lékařství*, 12 (1960) 347; *ibid.*, 13 (1961) 67.

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An automatic stepwise elution dispenser for column chromatography*

Certain studies in our laboratory are concerned with the separation of proteins and nucleotides. In these studies stepwise elution column chromatography is used. Reasons for this type of elution are to isolate given activities and to provide enriched fractions thereof. Since the time required to elute a given column is often in excess of 12-14 hours, it became necessary to construct an apparatus, that was not time dependent, to automatically allot the various buffers used.

Apparatus

Fig. 1 shows the dispenser we built to solve the problem. Eleven buffers can be accommodated with the machine (including one buffer in the reservoir).

The automatic dispenser was constructed so that each buffer is contained in a separatory funnel (Fig. 1). A tygon tubing is used to connect the respective funnels to the reservoir. Each tube is closed with a hospital type clamp. A stainless steel wire is used to connect each clamp to a respective solenoid, which at the proper time opens the clamp and allows the buffer to drain into the reservoir. The reservoir (Figs. 1 and 2) has a capacity of 500 ml. It is equipped with a glass float (b) connected to a glass rod to which a counter-balance (c) is used. At such time as the reservoir becomes empty, the level (bearing the counter-weight) is so devised to close switch (d). The closed circuit moves the motor driven contact arm (e) to the successive contact point which activates a solenoid. The solenoid activation results in opening the hospital type clamp through which the tygon tubing was placed. This then allows the buffer to drain into the reservoir. As the reservoir fills, switch (d) opens. This results in disconnecting the line voltage from the motor that drives the contact arm until such time as the reservoir again becomes empty.

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