Journal of Chromatography, 128 (1976) 302–303

CHROM. 9273

Note

Simple technique for the development of a dry column chromatogram

C. V. VISWANATHAN and V. K. LELEY
Lipid Research Laboratories, 8 Indrayani Flats, Prabhat Road Lane 15, Poona 4, 411004 (India)
(Received April 26th, 1976)

A chromatographic method termed dry column chromatography, with the resolving power of thin-layer chromatography (TLC) and the preparative capability of column chromatography, was first reported by Loev and Goodman[1]. A slight modification of this method, involving ascending development of the chromatogram, together with the use of mild aspiration to increase the velocity of migration of the developing solvent, was introduced by Jacini and Fedeli[2]. This modified technique was previously used by one of us in the preparative fractionation of complex microbial lipids (in the protozoan *Tetrahymena pyriformis* E)[3] and plant and animal lipids[4].

The use of a mixed solvent system for the development of a chromatogram gives a better resolution of complex lipid mixtures compared with a single solvent[5]. However, the application of mild aspiration[2] during the development of a chromatogram results in a selective and variable loss of the individual components of the developing mixtures, owing to their different vapour pressures. This effect changes the composition of the developing solvent mixture, affecting the subsequent separations. This difficulty has been overcome by the use of the simple technique described here.

EXPERIMENTAL

The construction of the apparatus is based on the principle of a liquid finding its own level in communicating containers, irrespective of their shape and size. A large test-tube (61 × 3 cm) serves as the chromatographic chamber and a uniform tube (61 × 2.5 cm), open at both ends, serves as the column. The column is packed as follows. One of its open ends is stoppered with a rubber cork (Fig. 1) and a small circle of filter-paper (radius 1.25 cm) is placed over the inside of the cork. TLC-grade silica gel (without binder) is added in small increments through a funnel while the column is rotated and tapped gently on the side. After each addition, the column is gently tapped vertically for a short period. This process is repeated until the column is filled to a height of 50 cm. On top of this column, a 5-cm layer of silica gel containing adsorbed lipid (5 g) is overlaid, this layer being covered with another 2-cm layer of plain silica gel. The remaining space in the tube is tightly packed with absorbent cotton-wool, completely filling the column. The chromatographic chamber, partly filled with the solvent mixture (Fig. 2a), is then held in an inclined position while the column is inverted, unstoppered and slowly inserted into the chamber. An initial floating of the column (*i.e.*, until it is partially wetted by the solvent) occurs within the chamber, and is due to the high density of chloroform, which is usually a major component of the mixed solvents used in the fractionation of polar lipids. This effect

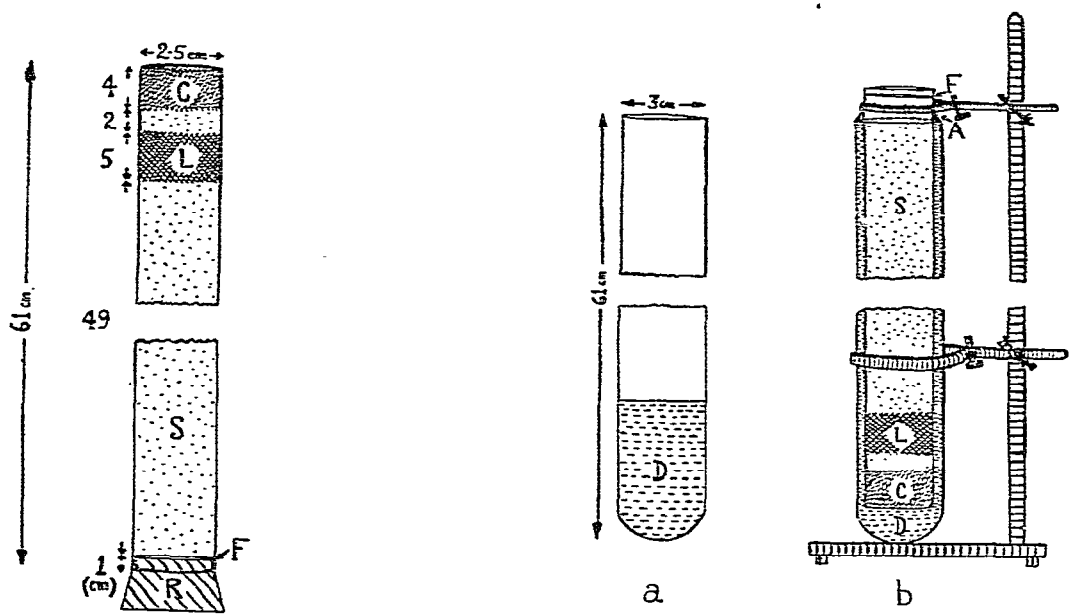

Fig. 1. Dry column filled with adsorbent. C, Absorbent cotton-wool; L, adsorbed lipid; S, silica gel (TLC grade); F, filter-paper; R, rubber cork.

Fig. 2. (a) Chromatographic development chamber partly filled with solvent mixture. D, Development solvent. (b) Ascending development of the dry column chromatogram. A, Aluminium foil; other symbols as in Figs. 1 and 2a.

is prevented by holding the column in place with a clamp at its free end, 2.5 cm above the mouth of the chamber. The chromatographic chamber is then filled with the same solvent mixture up to its brim and is replenished whenever necessary. Solvent evaporation by direct exposure to the atmosphere is prevented by covering the mouth of the chamber with aluminium foil. The column is then allowed to develop (Fig. 2b). The development time is comparable to that in the technique used previously[3].

DISCUSSION

This technique maintains a constant composition of a mixed developing solvent throughout the chromatographic process, and gives the desired separation of complex lipid mixtures. It can be used with advantage for a column of any dimensions, using a test-tube of corresponding size as the development chamber. At present, this technique is routinely used in our laboratory for the preparative fractionation of lipid mixtures. It should prove equally useful in other types of separations. Further, the equipment involved is very simple and inexpensive.

REFERENCES

1 B. Loev and M. M. Goodman, *Chem. Ind.*, (1967) 2026.
2 G. Jacini and E. Fedeli, *Advan. Exp. Biol.*, 4 (1969) 639.
3 C. V. Viswanathan and A. Nagabhushanam, *J. Chromatogr.*, 75 (1973) 227.
4 C. V. Viswanathan and A. Hayashi, *J. Chromatogr.*, 123 (1976) 243.