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Very long Z-shaped thin-layer chromatography and its application to the separation of dyes

Thin-layer chromatographic (TLC) separation of substances with R_F values about 0.1 calls for special procedures, such as the continuous flow method, which differs only from the normal technique in that the development time can be made as long as required. However, if low polarity components are present in the initial mixture, they will migrate to the end of the plate and as a result of this they will appear mixed together with the solvent and plate impurities in the case of the ascending procedure, or they will be washed off the plate in the case of descending chromatography.

Although these difficulties can be overcome by employing very long plates¹, special equipment is required for their handling. However, it is also possible to increase the actual length of the development zone considerably by the suitable arrangement of the adsorbing material on commercial size plates.

To this end we have used TLC plates from which we have partially removed the adsorbing layer in order to form a zigzag development path with the remaining adsorber; development zones up to 2 m long can be made this way from 20×20 cm plates, the length depending on the width of the development zone.

Experimental and results

The shape we have adopted can be seen in Fig. 1. Particular attention has been

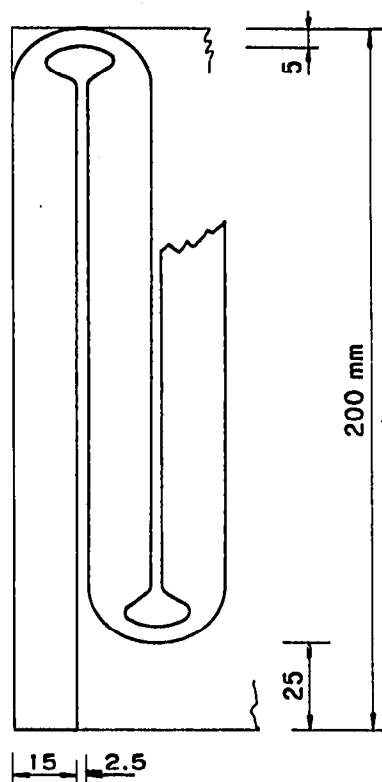


Fig. 1. Arrangement of the adsorbent on the plate.

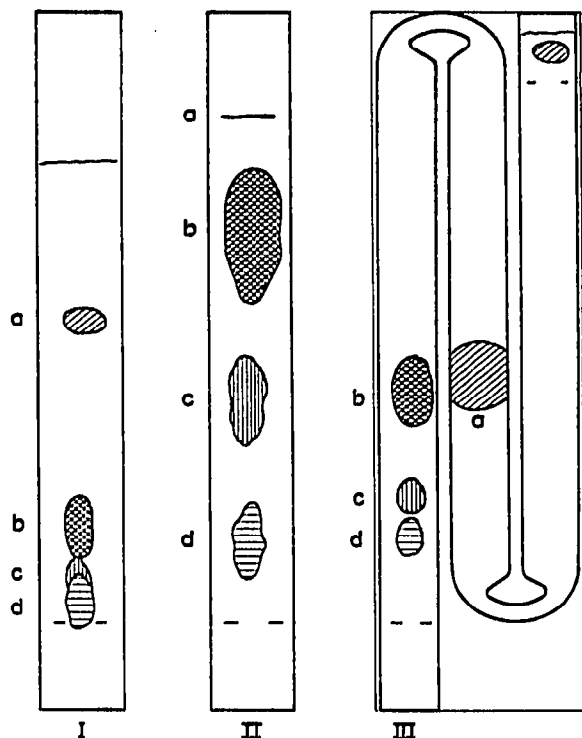


Fig. 2. Comparison of the present technique with other TLC methods. I normal; II continuous flow; III Z-shaped. (a) dimethyl yellow; (b) rhodamine B; (c) methyl red; (d) 2',7'-dichloro-fluorescein.

paid to the bent portions in order to ensure that the distance traveled at both edges of them is almost the same, otherwise the spot length would increase at each turn. It has been found experimentally that 5 mm is a suitable width at corners for straight development zones 15 mm wide, although other widths can be used, according to GRIESEMER², because the influence of this dimension on R_F values is slight.

The scraped portions of the plate shall be cleaned very carefully in order to avoid solvent transfer from one strip to the next through residual particles of adsorbent. A few minutes only are needed to prepare a plate if a template is used for drawing the path and as a guide for removing the excess of adsorbent.

As a demonstration of the advantages of this procedure as compared with other methods, the separation of four dyes (dimethyl yellow, methyl red, 2',7'-dichloro-fluorescein and rhodamine B) has been carried out with acetone as solvent, on pre-coated silica gel plates $20 \times 20 \times 0.025$ cm (Merck, ref. 5715), in cylindrical tanks 5.5 cm diam. and 21 cm high with saturated atmosphere. The results achieved with three different separation procedures can be seen in Fig. 2; these procedures are as follows:

(a) Normal ascending chromatography, development time 30 min: only dimethyl yellow is separated from the other dyes.

(b) Ascending chromatography with continuous flow: the procedure described by BOBBITT³ has been employed. A continuous flow of the solvent is maintained by passing the top of the plate through a slot in the tank lid. After a development time of 4 h 30 min, the four dyes were separated but dimethyl yellow is found at the front along with the solvent and plate impurities.

(c) Very long, Z-shaped chromatography: a plate 50 mm wide with an effective adsorbent width of 14 mm is used; the actual length of the development zone is 56 cm. Contact of the adsorbent with the saturating paper wick is avoided by scraping a zone 1 mm wide at each side of the plate. After 5 h 30 min of development time, a good separation of the four dyes is achieved with the advantage that dimethyl yellow can be recovered free of impurities. It becomes increasingly difficult to visualize the position of the solvent front along the path; therefore, in order to know when the solvent front arrives at the end of the path or at any other place, it is advisable to apply a small amount of a low-polarity dye, such as dimethyl yellow, at this point. Its development points out the end of the experiments.

The separated substances can sometimes be identified by the normal methods of TLC to find out their relative positions; at other times, an independent Z-shaped chromatogram will be needed in order to determine the R_F value of a standard. We have used the first method to distinguish between dimethyl yellow and methyl red; the other two dyes are fluorescent and they have been identified with UV light.

R_F values obtained by this procedure are lower than those from normal TLC because the dry adsorbent is excessively exposed to the vapors of the solvent; this is equivalent to saturating the adsorbent previously with solvent vapor⁴.

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