Precision Zone Detection in Thin-Layer Chromatography

Sidney K. Wolfson, Jr.,* John C. Lydon, Shang J. Yao, and Richard W. Keller, Jr.

Department of Neurological Surgery, University of Pittsburgh, and Surgical Research Laboratory, Montefiore Hospital, Pittsburgh, Pennsylvania 15213

Recovery of the various components separated on a thin-layer chromatography (TLC) plate can present a problem because of uneven front migration which can occur on any particular trial for a variety of reasons. One of the more common causes is the use of TLC as a preparative method requiring heavy loading of the plate with sample in an effort to recover the maximum amount possible (*1*). Another cause is the presence of large concentrations of extraneous solute such as electrolyte that cannot be removed by pretreatment (e.g., ion exchange). This cause of uneven migration is a particular problem in the TLC separation of compounds which have been preconcentrated by evaporation in physiologic buffers. We report here a method and apparatus for precisely tracking the irregular component fronts without contaminating them with staining reagents. Thus, the bulk of the developed chromatogram can be separated and eluted.

The method involves the use of a "slitted mask" (Figure 1), a sheet which covers the entire surface (20 × 20 cm) of the thin-layer chromatography plate. The mask consists of a sheet of polystyrene (1-mm thick) with 2-mm slits regularly spaced at 22-mm intervals. The slits run perpendicular to the sample streak and extend nearly the entire length of the sheet. The sample is streaked on the plate and developed in the usual fashion. When the solvent has evaporated from the plate, the mask is placed over it and secured by a frame clamped at the top and bottom. A visualization reagent specific to the components being separated is then lightly sprayed over the surface of the mask. Because of the pressure exerted by the frame, the mask is held in contact with the plate and only the surface directly under and exposed by the slits is contacted by the reagent. When the color is developed, the mask is removed. Spots or dashes are seen along each product front where it was exposed by the slits. The upper and lower edges of the spots are connected by a scratch or light pencil line to outline zones (Figure 2). The adsorbent with sample is then scraped off and eluted in an appropriate solvent.

There are several advantages to this method. The number of slits stained may be increased if migration is particularly irregular, thus yielding more stained spots that follow the irregular contour more clearly. There is no excess adsorbent scraped into each fraction, only that which contains sample material. This is an important consideration when using an eluting solvent which causes partial dissolution of the adsorbent or when dealing in micro quantities. Guesswork and dependence on previously calculated R_f values are eliminated. Separations need not be extensive since partial visualization is possible using our method. With the mask described above, it was determined on the basis of area calculations that only 8% of the solute would be lost through interaction with the visualization reagent. Naturally the amount of solute lost during visualization is dependent upon the slit width and the number of slits.

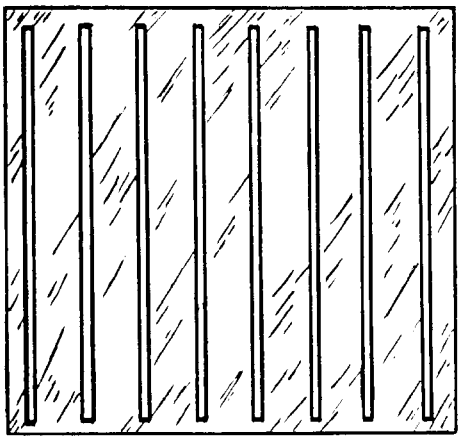

Figure 1. A polystyrene slitted mask

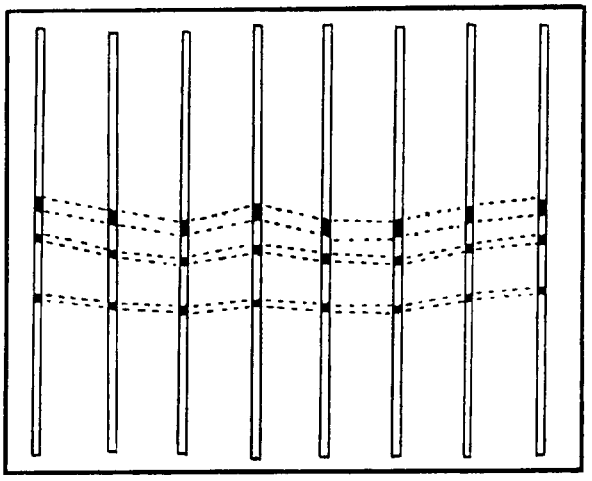

Figure 2. Separation of mixture of fructose, sucrose, and lactose in TLC with the use of a slitted mask

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LITERATURE CITED

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