

June 15, 1971

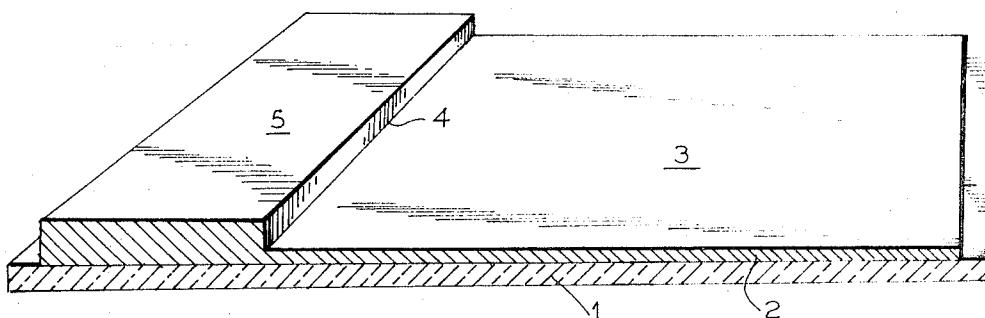
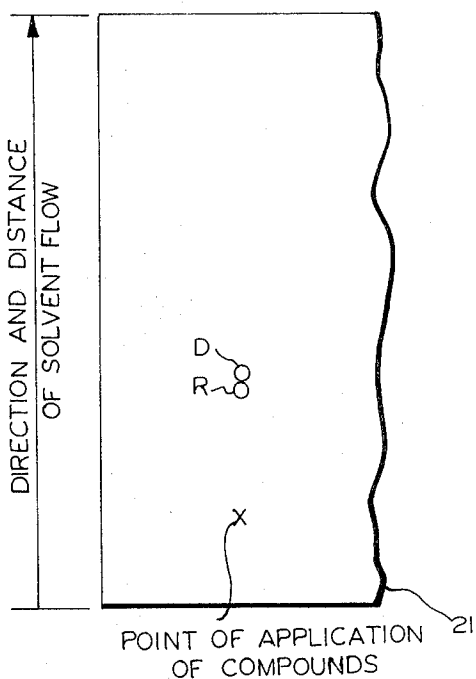
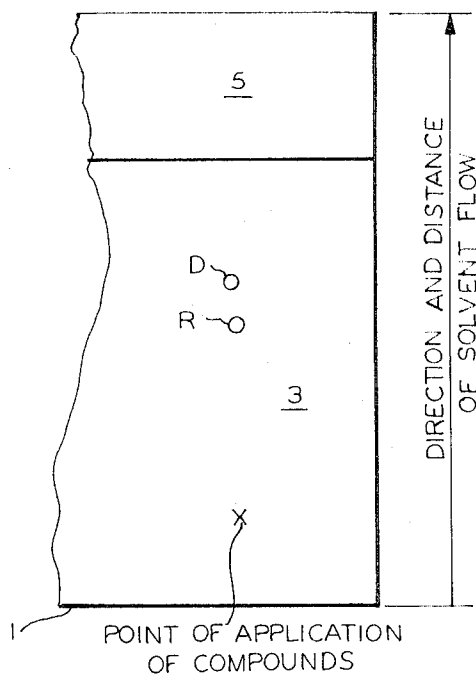
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3,585,129

DUAL THICKNESS TLC PLATES AND DEVICE THEREFOR

Original Filed Nov. 14, 1967

3 Sheets-Sheet 1



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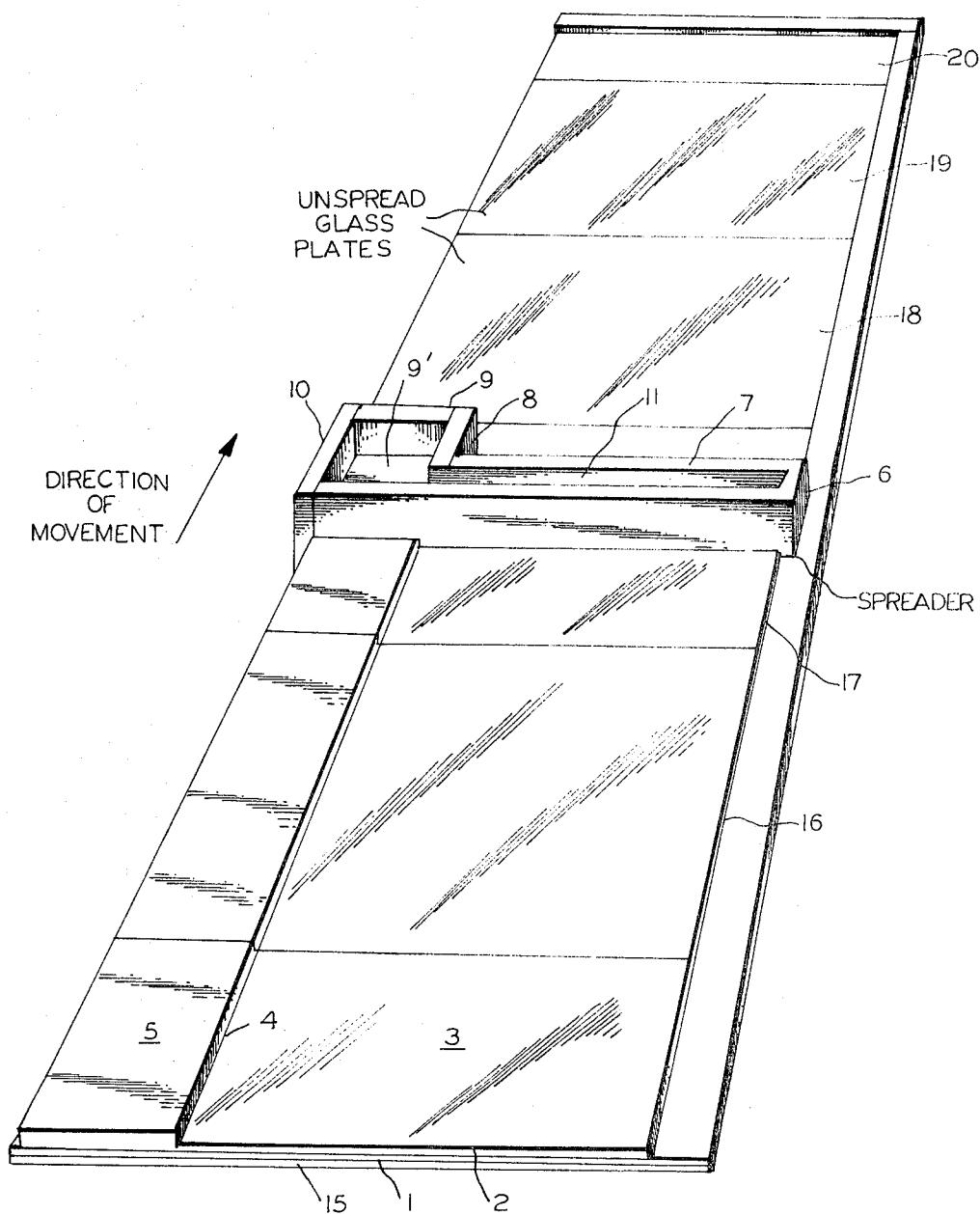
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DUAL THICKNESS TLC PLATES AND DEVICE THEREFOR

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DUAL THICKNESS TLC PLATES AND DEVICE THEREFOR

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3 Sheets-Sheet 3

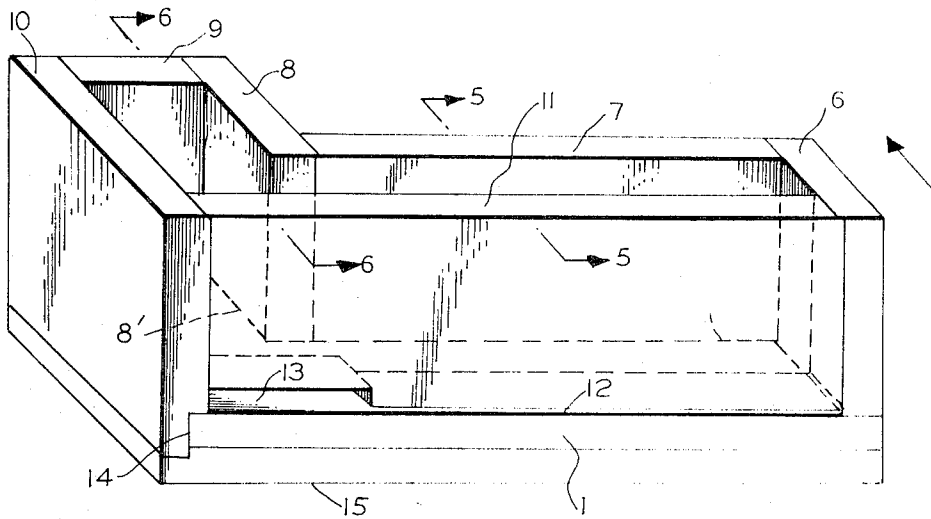


FIG. 4

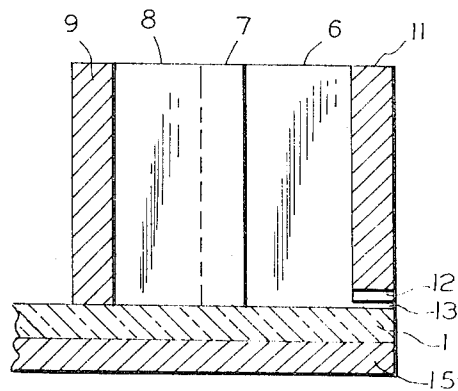


FIG. 6

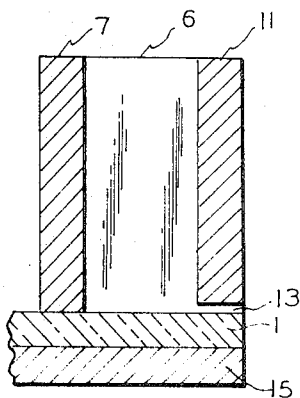


FIG. 5

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1

3,585,129

DUAL THICKNESS TLC PLATES AND  
DEVICE THEREFORNorman E. Delfel, Peoria Heights, Ill., assignor to the  
United States of America as represented by the Secretary of AgricultureOriginal application Nov. 14, 1967, Ser. No. 683,439, now  
Patent No. 3,522,792, dated Aug. 4, 1970. Divided  
and this application Mar. 28, 1969, Ser. No. 835,835

Int. Cl. B01d 15/08, 15/06

U.S. Cl. 210—198C

1 Claim

## ABSTRACT OF THE DISCLOSURE

Improved TLC resolutions are obtained on plates on which the thickness of the continuous adsorbent layer is abruptly increased by a factor of about four or five to provide a narrow, deeply coated zone that increases the uptake of solvent. A spreader for readily preparing the dual thickness plates also is described.

A non-exclusive, irrevocable, royalty-free license in the invention herein described, throughout the world for all purposes of the United States Government, with the power to grant sublicenses for such purposes, is hereby granted to the Government of the United States of America.

This application is a division of Ser. No. 683,439, filed Nov. 14, 1967, now U.S. Pat. No. 3,522,792.

This invention relates to the discovery that the thin-layer chromatographic (TLC) resolution of very difficultly separable isomers is remarkably improved by employing a properly oriented TLC plate of conventional length, i.e., ca. 20 cm. upon which the coating or layer of siliceous adsorbent has been so deposited as to provide a relatively wide major zone whereof the coating has a uniform depth or thickness of 0.25–1.0 mm., and in abutting continuity therewith a much narrower zone whereof the depth of the deposited adsorbent is about four or five times that of the other zone.

Conventional TLC separations of poorly separable compounds, e.g., a mixture of rotenone and deguelin or of isomeric unsaturated fatty acids or fatty esters on 20 cm. planar glass plates coated with a precisely deposited uniformly thin layer of silica gel adsorbent often fails to provide sufficient resolution of the isomers.

It is known that such inadequate resolutions can sometimes be greatly improved merely by using coated plates having a length of at least 40 cm., which increase the amount of solvent travel, or by applying streamers of adsorbent paper to the uppermost portion of the siliceous coating on a standard plate. However, the overly long plates are more costly and fragile and are cumbersome both to prepare and to use, whereas the streamer-fortified plates fail to show the position of the solvent front, which positional information is used to avoid either insufficient or excessive exposure of the plate to the solvent prior to the use of the color developing reagent.

The present invention resides in the discovery that the superior TLC resolutions that are presently obtained on conventionally coated plates that are at least about 40 cm. in length can be obtained with 20 cm. plates that are uniformly coated with a conventionally thin (0.25–1.0 mm.) layer of dried silica gel adsorbent excepting along a marginal portion of the length of the glass plate where the depth of the deposited coating has been abruptly increased by a factor of four or five to provide an immediately adjacent and continuous, parallel, deeply coated zone that constitutes 0.2–0.25 (5–4 cm.) of the length of the plate. The spotting of the resulting dual zone TLC plate with the mixture to be resolved is performed after

2

the plate is so oriented (90° rotation) that the relatively narrow zone or band of deeply applied adsorbent is up (furthest from the pool of solvent) whereas the thinly coated major zone dips thereinto.

For maximum efficiency, the widths of the respective zones should be inversely related to the respective thicknesses of the adsorbent in the two zones, and vice versa.

The invention also relates to a novel spreader for simultaneously depositing the adsorbent in the form of two contiguous zones characterized by inversely related and distinctly different widths and depths of adsorbent, whereby is produced my improved dual zones TLC plate. In general, the spreader comprises a vertically walled, otherwise open enclosure having a reversed open block L-shaped configuration, which enclosure becomes a bottomed reservoir or hopper when the empty enclosure is placed at the margins of a gap-free series of the uncoated plates (which temporarily serve as a bottom for the enclosure in addition to being the substrate to be coated). When the reservoir is filled with a slurry comprising a siliceous adsorbent, e.g., purified silica gel, and is manually advanced to the opposite end of the series of glass plates, there are deposited simultaneously, and in only single transit, both the wide normal depth band and immediately adjacent thereto a correspondingly narrowed but much more deeply coated band.

Although a more detailed description of the adsorbent spreading device for preparing my novel dual zone TLC plates will be given in connection with the drawings, the device may be broadly described as being a somewhat L-shaped, vertically-walled enclosure, a functional bottom for which is temporarily provided by placement upon the plate that is to be coated. The lower (functionally trailing) edge of the longest wall is raised above the plate just enough to permit depositing a conventionally thin layer of silica gel excepting for a minor (relatively narrow) terminal segment where the edge of the spreader is abruptly further raised so as to form a gap that is capable of depositing preferably about four times as great a depth of adsorbent, the polydimensional gap thus being capable of simultaneously forming a wide major zone of adsorbent having the usual TLC depth and, in continuous side-by-side adjacency thereto, a much narrower but correspondingly more heavily deposited strip or zone. To provide a properly increased supply of silica gel adsorbent for the more deeply layered zone, the hopper is suitably enlarged across from but directly in line with the more widely open gap segment, thereby giving the L-shaped configuration of the hopper.

With reference to the drawings, FIG. 1 represents an isometric view of a dual-zone TLC plate according to the present invention. FIG. 2 represents an isometric view of the partially emptied dual discharge hopper of the invention in place on the surface of a series of side-by-side glass plates over which it has completed a partial transit. FIG. 3A is a representation of the complete resolution of the rotenone-deguelin mixture referred to in column 3 hereof using the dual zone TLC plate of the invention while FIG. 3B is a representation of the overlapping resolution obtained on a conventionally coated 20 cm. TLC plate. FIG. 4 is an isometric view of the applicator, FIG. 5 is a section taken along lines 5–5 of FIG. 4, and FIG. 6 is a section taken along lines 6–6 of FIG. 4.

As seen in FIG. 1, the major portion of glass plate substrate 1 is coated with a uniformly thin layer 2 of siliceous adsorbent to provide thin layer zone 3 which at demarcation line 4 is abruptly thickened to provide thickly coated narrow zone 5.

Referring to FIG. 2, it will be seen that the hopper comprises six successively integral vertical walls, respectively, 6, 7, 8, 9, 10, and 11, which together define an

3

enclosure having a configuration approximating that of a block letter upper case L. Wall 11 is the trailing wall when the spreader is moved across the plates and is distinguished by its failure to extend downward quite to the reference plane of the glass that is to be coated and also to the plane of the lower edges of walls 6, 7, 8, and 9 (shown as 6', 7', 8', and 9') whereby are formed, with reference to the said plane, a nearly full width standard aperture or gap 12 and continuous therewith, abruptly widened short segment aperture 13. This is shown in FIGS. 5 and 6. Vertical wall 10 not only contributes to the formation of said segmental aperture but also extends further to define flange like edge 14, which extends below the glass plate and serves as a guide or stop for maintaining the spreader device in position on any suitable flanged support 15.

Although I prefer a 4:1 ratio of the respective gap or aperture widths with inverse 1:4 ratios of gap heights, the invention is not strictly limited to these values which will be influenced by the particular compounds to be separated, the adsorbent to be deposited on the glass plates and the solvent system.

Those skilled in the preparation of uniformly coated TLC plates will recognize the impracticability of making one or two plates at a time and are accustomed to place about a half-dozen or so uncoated plates in successive side-by-side contact on flanged support 15 and then progressively coating the series of plates with a uniformly thin layer of adsorbent by moving the spreader only once from one end of the series to the other. The dual zone spreader of the invention is employed in an identical manner to provide a plurality of dual zone TLC plates 16, 17, 18, 19, and 20 as seen from FIG. 2.

#### Example

To illustrate the beneficial operativeness of my dual zone TLC plates, 10 microliter amounts of an acetone solution containing per milliliter 1 mg. of rotenone and 1 mg. of deguelin (designated as R and D, respectively in FIGS. 3A and 3B) were spotted 3 cm. from the bottom of each of two 20 cm. x 10 cm. TLC plates, one plate 21 being conventional in having been uniformly coated with a subsequently dried 0.25 mm. thickness of a silica gel slurry in 12% AgNO<sub>3</sub> (1 g. silica/2 ml. of the

4

AgNO<sub>3</sub> solution) whereas the other plate was identical with respect to the presence of a 0.25 mm. depth silica gel major zone but differed in that the deposit of silica gel on the last 5 cm. of the 20 cm. length of the plate furthest from the sources of solvent was abruptly increased by a factor of four to a depth of 1 mm. The plates were exposed for one hour to a solvent system consisting of chloroform containing 2.5% acetone and 0.5% acetic acid, the conventionally (0.25 mm.) coated end of the dual zone plate being introduced into the solvent mixture. Then to develop the color, the plates were in turn exposed first to HNO<sub>3</sub> vapor and then to NH<sub>3</sub> vapor. On the conventional TLC plate the centers of the two spots were only 0.9 cm. apart, and the resulting overlap prevented effective use of an optical densitometer. By contrast the 1.5 cm. center-to-center distance of the developed spots on the dual zone plate avoided overlapping of the spots and permitted an accurate quantitation.

I claim:

1. A silica gel coated dual zone TLC plate capable of providing improved resolutions of rotenone-deguelin mixtures, said plates being characterized by an abruptly demarcated but continuously integral and independently planar zone having a 4- to 5-fold greater depth of silica gel coating in an area confined to 1/4-1/3 of the length of the plate.

#### References Cited

##### UNITED STATES PATENTS

|           |         |                 |         |
|-----------|---------|-----------------|---------|
| 3,457,897 | 7/1969  | Warren          | 118—413 |
| 3,289,241 | 12/1966 | Garrison et al. | 118—415 |
| 3,203,021 | 8/1965  | Pelick          | 118—413 |
| 3,145,410 | 8/1964  | Stahl           | 118—413 |

##### OTHER REFERENCES

Truter, E. V.: Thin-Layer Chromatography, London, Cleaver-Hume Press Ltd., 1963, pp. 13, 33, 34.

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U.S. Cl. X.R.

117—37R, 43, 169