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 [33] **Japan**
 [31] **43/70078**

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[54] **CONTINUOUS FLOW, PREPARATIVE THIN-LAYER CHROMATOGRAPH**
8 Claims, 10 Drawing Figs.

[52] U.S. Cl. **210/31**
 [51] Int. Cl. **B01d 15/00**
 [50] Field of Search..... **210/31, 198**

ABSTRACT: An apparatus for thin-layer chromatography which comprises a support-coated glass plate, cover plates which are same in width as said glass plate and shorter in length than said glass plate and have spacers with same length on their both sides, clips for fitting said glass plate with said cover plate so that the latter plate can be moved up and down, and a solvent container with a lid into which the lower ends of both said glass and cover plates can be immersed.

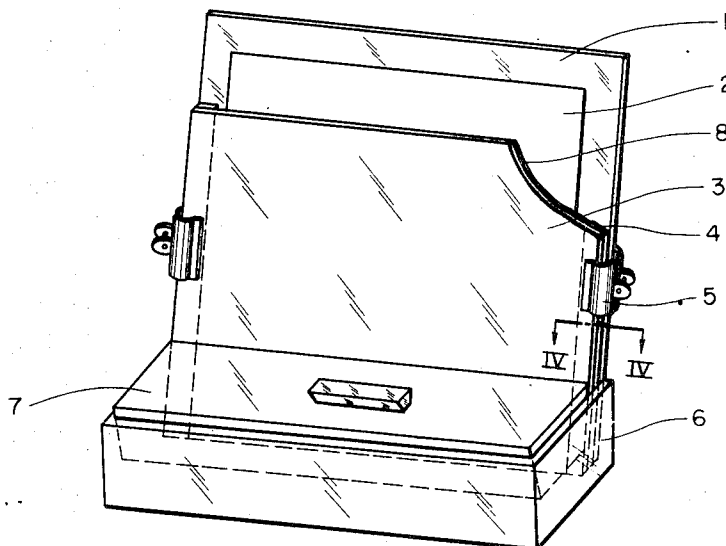


FIG. 1

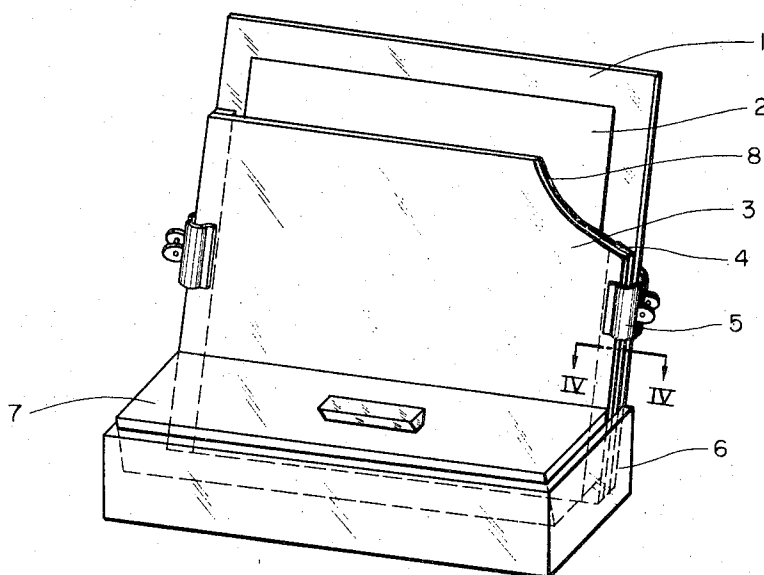
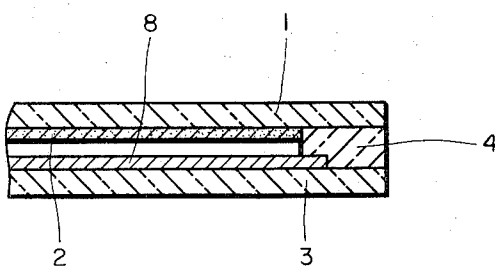


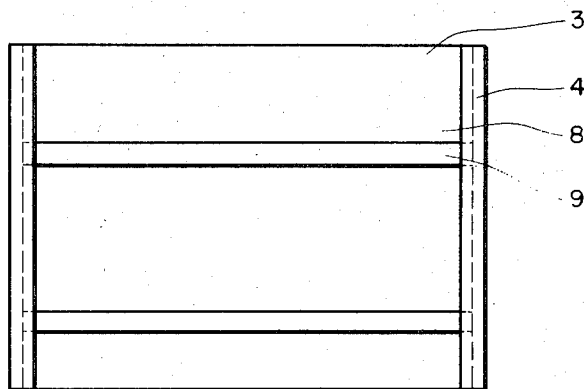
FIG. 2



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FIG. 3



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FIG. 4

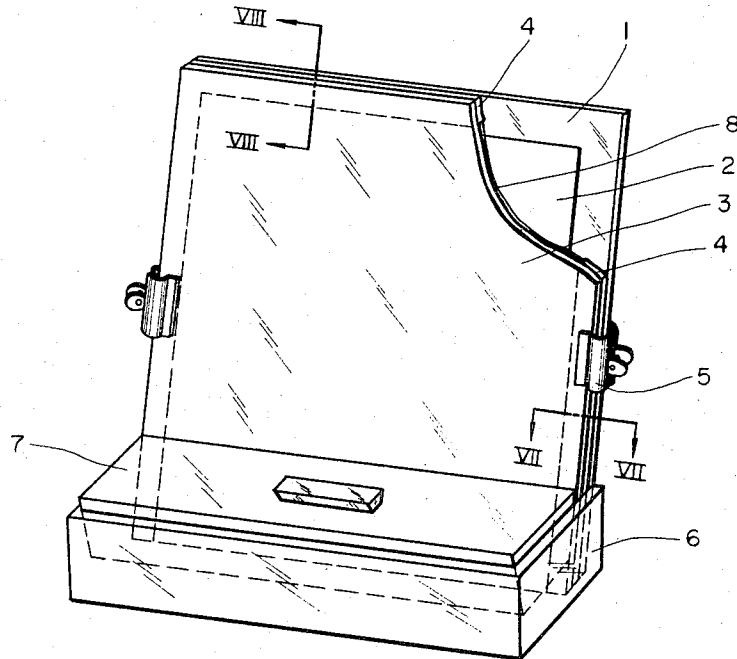


FIG. 5

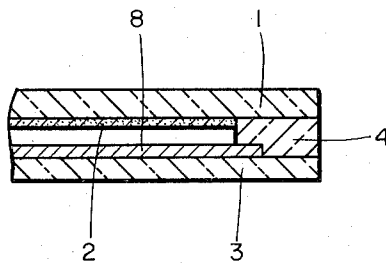
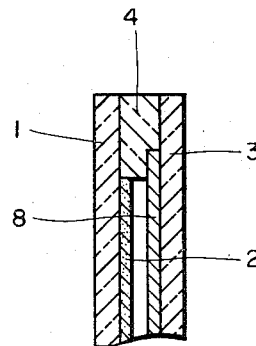


FIG. 6



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FIG. 7

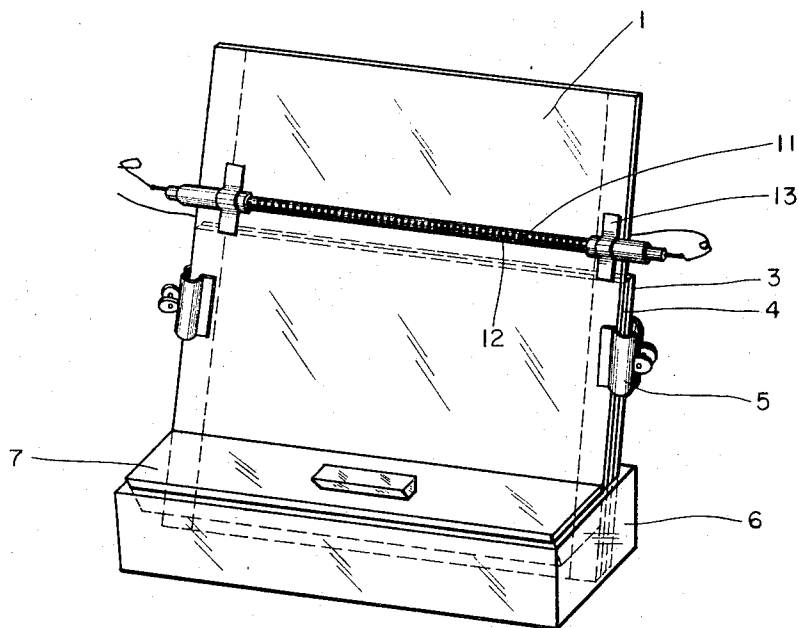


FIG. 8a

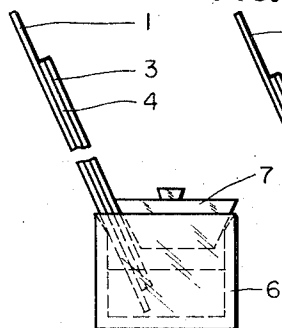


FIG. 8b

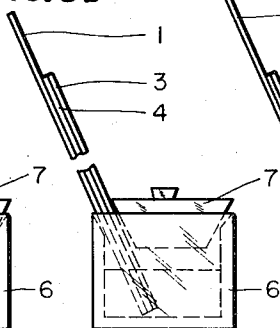
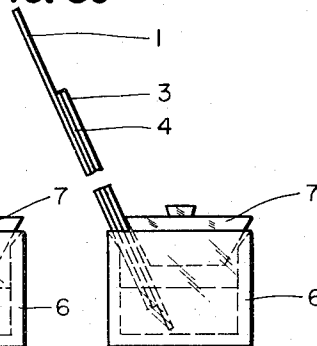


FIG. 8c



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CONTINUOUS FLOW, PREPARATIVE THIN-LAYER CHROMATOGRAPHY

BRIEF SUMMARY OF THE INVENTION

The present invention relates to an improved sandwich-type apparatus for thin-layer chromatography which can separate and condense sample components in lines by the continuous flow evaporation and, more particularly, to an apparatus for thin-layer chromatography which comprises a support-coated glass plate, cover plates which are the same in width as said glass plate and shorter in length than said glass plate and have spacers with the same length on both sides, clips for fitting said glass plate with said cover plate so that the latter plate can be moved up and down during chromatography, and a solvent container with a lid into which the lower ends of both said glass and cover plates can be immersed.

An object of the invention is to provide an apparatus for thin-layer chromatography which is effected by a much shorter presaturation of the chromatographic chamber with solvent vapor and separates and condenses sample components continuously at appropriate areas of the chromatographic plate without influencing the vapor saturation in the developing chamber.

A further object of the invention is the provision of an apparatus for thin-layer chromatography which gives reproducible chromatograms and a good separation of the samples.

Another object of the invention is the provision of an apparatus for thin-layer chromatography which enables easy recovery of sample components from a thin-layer plate by the elution with solvents.

A still further object of the invention is the provision of a thin-layer chromatographic apparatus of such nature that it prevents the solvent demixing which occurs in a thin-layer during the development when a solvent mixture containing polar solvents is used as a developing system.

A still further object of the invention is the provision of an apparatus for thin-layer chromatography which is capable of rapid chromatographic development and minimizes the diffusion of sample spots separated during the development.

Other objects of the invention will be obvious from the contents of the specification hereinafter disclosed.

CROSS-REFERENCES TO RELATED APPLICATION

Various methods have been proposed for preparative thin-layer chromatography of a sample mixture applied in line. However, they are not favorable in view of giving diffused and distorted chromatographic bands of the sample components. For removal of these defects, Mulder and Veenstra have provided a device involving the evaporation of developing solvents from the space at the top of the horizontal developing chamber, by which bands of the sample components are resolved and narrowed. And then, they developed the chromatogram obtained thereby in the second-dimensional direction in order to condense the bands as spots (*J. Chromatogr.*, 24, 250 (1966)). However, their method is not only so complicated that it could not be practical, but it is difficult to condense the sample components continuously. The continuous flow-developing method would be applicable to the box-type developing chamber proposed by Truter (*J. Chromatogr.*, 14, 57 (1964)), as another device for example. However, it could not be utilized for the purpose of the stepwise condensation of the sample components without placing a mount in the solvent container to raise coated plate. Consequently, that may influence the magnitude of saturation of solvent vapor and result in worse resolution of the chromatographic bands. Change in the solvent vapor saturation during the development, in general, remarkable influences chromatographic mobilities of samples and poor resolution of mixture follows and frequently inversion of their R_f values.

The sandwich-type developing chamber available on the market consists of a cover plate with a spacer on its edge except the lower side, a glass plate with the same size as that of the cover plate and coated with an adsorbent layer except the

area where the spacer contacts, and a solvent container. When it is used, the glass plate is fitted with the cover plate, and then the lower ends of both glass and cover plates are immersed in a developing solvent places in the solvent container. However, slow soaking-up of the developing solvents and low separability of the sample mixture have given the apparatus much disadvantage.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

FIG. 1, FIG. 2 and FIG. 8 show oblique views of another type in this invention and an enlarged sectional view at the line IV—IV in FIG. 1, respectively.

FIG. 3 shows a cover plate on which a porous medium is attached and pressed down from both sides of the cover plate by slender springs.

FIG. 4 shows an oblique view of another embodiment of this invention and FIG. 5 and FIG. 8 show enlarged sectional views at the line VII—VII and VIII—VIII in FIG. 4, respectively.

FIG. 7 shows an electric heating means which is fixed on the glass plate so that the means can be moved up and down.

DETAILED DESCRIPTION OF THE INVENTION

This invention overcomes the above-mentioned defects, through an apparatus for thin-layer chromatography which includes a support-coated glass plate, cover plates which are same in width as the glass plate and shorter in length than the glass plate and have spacers with the same length as the cover plate on both sides and are used for covering the glass plate, clips for fitting the glass plate with the cover plate so that the latter plate can be moved up and down, and a solvent container with a lid into which the lower ends of both said glass and cover plates can be immersed.

However, the chromatographic apparatuses have been found to cause solvent demixing in the adsorbent layer when solvent mixtures containing polar solvents are used as developing systems. The above is based on the different adsorptivities of the solvents used and leads to decrease in reproducibility of the chromatograms, giving different results from those obtained with the usual box-type chromatographic apparatuses.

The inventor has made further investigations to eliminate the solvent demixing and reached a conclusion that the defects mentioned above are overcome by attaching filter paper, glass fiber sheet, or any other porous material, incapable of absorbing polar solvents, inside the cover plate not as to come into contact with the adsorbent layer and then impregnating it, prior to the development, with a solvent mixture to be used. Treatment of the cover plate as to give a porous inside is also as effective as the above device.

The invention will be readily understood from the following detailed description.

Referring now to FIG. 1 and FIG. 2, the glass plate 1 is coated with an adsorbent layer on the inside. The cover plate 3, made of glass, plastics or metals whose width is same as that of the glass plate 1 and length is shorter, has glass, plastic or metallic spacers 4 on both sides which are thicker than the gel layer, viz 1–5 mm., preferably 2–3 mm. The spacers 4 have even faces to prevent solvent vapor from leaking. By clips 5 the cover plate 3 is fitted on the gel-coated glass plate 1, and their lower ends are immersed in a developing solvent places in a container 6. Loosening the clips 5, the cover plate 3 can be worked up and down at any appropriate position on the glass plate 1. 7 is a lid for the solvent container 6.

FIGS. 3, 4 and 5 also show an apparatus equipped with a porous medium 8 attached between the spacers 4 shown in FIG. 1, which consists of filter paper, glass fiber sheet, or any other porous medium 8 incapable of adsorbing polar solvents. As shown in FIG. 4, for attaching the porous medium 8 inside the cover plate 3, the use of spacers 4 with grooves in their insides and pressing down the porous medium 8 by slender springs 9 is recommended because they can prevent the

porous medium 8 swollen with solvents from slackening and coming into contact with the adsorbent layer 2. Instead of the use of the cover plate 3 attached with the porous medium 8, a cover plate with a porous surface structure on its inside face may be useful. In this case, there is no need to use the grooved spacers. Thickness of the spacer should be 1-5 mm. more favorably 2 to 3 mm.; thickness less than 1 mm. and more than 5 mm. is unfavorable because in the former, the developing solvent comes up through capillary action and in the latter, saturation of the adsorbent layer with solvent vapor needs longer time.

FIGS. 6, 7 and 8 represent a cover plate 3 with spacers 4 having the same thickness as those shown in FIG. 1, on both sides, and the numerals corresponds to those in FIG. 1.

In FIGS. 1, and 6, the cover plate is equipped to cover the slant glass plate 1 faced upward, for convenience of explanation. The cover plate may also be used to cover the glass plate faced downward. In the latter case, the porous medium attached inside the cover plate is prevented more effectively from coming into contact with the adsorbent layer. And also the plates may be used vertically.

Furthermore, FIG. 7 shows an electric heating means 10 equipped on the upper side of the glass plate 1 of the opposite side of the cover plate 3 as shown in aforesaid figures, so that the heating means 10 may be worked up and down at any appropriate position where sample components are condensed. The heating means 10 comprises a glass tube 11, an electric heating wire 12 made of a nickel-chrome alloy inclosed in the glass tube 11, and the wire is connected to an electric source. Number 13 shows adhesive tape retaining heating unit 10 on the glass plate 1.

The operation, uses and advantages of the present device are as follows:

As an example of the operation and use of the present apparatus, the development of a mixture of three dyes is described (butter-yellow, Sudan-red G, and indophenol).

A solution of 2 mg. each of the three dyes in 0.1 ml. of benzene was applied in a line to the lower end of a silical gel-coated glass plate 1 (20×20 cm. in size), and as illustrated in FIG. 10a) the lower end of the plate 1 was then immersed in benzene placed in the solvent container 6 after being covered with the cover plate 3 (20×24 cm. in size) using clips 5. The developing solvent, benzene, migrated the dyes to give horizontally linear bands with different mobilities during soaking up in the gel layer and was evaporated when it reached the position corresponding to the upper end of the cover plate 3. Complete resolution of the dyes was achieved in 20 min. after the development, and the band of butter-yellow with the largest chromatographic mobility reached the top of the cover plate 3 and, according to the evaporation of the developing solvent, turned into a very narrow and condensed band about 40 min. later. After that the cover plate 3 was moved down to the position of 5 mm. underneath the condensed band of butter-yellow by taking off and refitting the clips 5, and then the development was continued as illustrated in FIG. 8). During the procedure to move down the cover plate 3, little possibility of change in the magnitude of the vapor saturation was there, and consequently, no disturbance was caused in chromatographic resolution of the dye bands. The band of Sudan-red G was also developed to the top of the cover plate in 4 hrs. and condensed as a very narrow band. Then the cover plate 3 was moved down to the position of 5 mm. underneath the condensed band of Sudan-red G, and the development was continued about 1 hr. more as illustrated in FIG. 8) until the band of Indophenol reached the top of the cover plate and was condensed.

While only one embodiment of the device has been described in detail, it is particularly understood that the device is not limited thereto and thereby, but can be applied to the resolution of mixtures consisting of more than three components. In this case, when the lower end of the glass plate 1 can not be immersed in the solvent by moving the cover plate 3, the latter plate is replaced with a shorter one for further chromatographic procedures.

As a summary; (1) The recovery of each component of a mixture is very readily established by making use of the present apparatus. (2) Samples can be chromatographed without presaturation of the thin-layer plate with solvent vapor, and which does not cause any disturbance of their chromatographic mobilities, permitting fairly good reproducibility of the results and easy operation. (3) It is easy to alter the area for condensing the sample band without disturbing the saturation of solvent vapor. (4) The apparatus can be utilized not only when any type of chromatographic supports coated in various thickness are used, but also rather efficiently for the usual purpose of thin-layer chromatography because of its advantage that the presaturation with solvent vapor is unnecessary.

Referring again to - FIG. 1 for further explanation about the practical use of the present apparatus, after application of a solution of sample mixture to the lower side of the thin-layer 2 coated on the glass plate 1, the cover plate 3 fitted with a porous medium 8 is equipped using clips 5 to cover the adsorbent layer, and prior to the development, only the lower end of the porous medium 8 is immersed in a developing solvent mixture places in the solvent container 6 to saturate the thin-layer 2 with solvent vapor. Though the time needed for the saturation differs according to nature of the solvent mixture used, it is usually only a few minutes. After that, the cover plate 3 is moved up a little by loosening the clips 5 and then tightening them, the both ends of the glass plate 1 and the cover plate 3 are immersed in the solvent mixture. The solvent mixture migrates the components of sample during soaking up in the thin layer and is evaporated after reaching the top of the cover plate 3. After an appropriate interval, the band of the component with the largest mobility reaches the top of the cover plate 3 and condenses there in a line.

After the complete condensation of the first developed band, the clips 5 are loosened and the cover plate 3 is moved down at a small distance from the line of the first component. Tightening the clips 5, the development is continued until the second component is condensed at the top of the cover plate 3. Repetition of the above procedure leads all components of the mixture to the complete separation and condensation in lines at appropriate positions.

The apparatus illustrated in FIG. 4 used in the same way as described above for the one in FIG. 1. Prior to the development, the cover plate 3 is fitted on the glass plate 1 with the clips 5 so that only the lower end of the porous medium attached inside the cover plate can be immersed in the developing solvent mixtures. The time needed for the saturation with solvent vapor is almost same as in the former apparatus. After immersion of both ends of the cover plate 3 and the glass plate 1 in the solvent mixture, the development is carried out. According to soaking up of the solvent in the thin layer, the sample applied to the lower side of the layer is chromatographed.

The use of the present apparatus as shown in FIG. 1 and 4 gives more advantages in comparison to the ones available on the market. From the viewpoint of accelerating the evaporation of less volatile solvents, a further advantage can be obtained by the use of the heating device shown in FIG. 7.

a. They establish rapid development, prevent diffusion of spots or bands of the sample components, and give well-resolved chromatograms with high reproducibility.

The above will be demonstrated by the following examples which were obtained with a silica gel thin-layer plate with a thickness of 250 μ prepared in the usual manner. The development was carried out 10 cm. from the lower end of the plate.

Time for 10 cm. development (min.)		
Solvent	Solvent	
$C_6H_6-Me_2CO(4:1)$	$C_6H_6-MeOH(9:1)$	
The present apparatus in FIG. 1	18 $\pm$ 2	19 $\pm$ 2

The present apparatus	21 $\pm$ 2	22 $\pm$ 2
A usual sandwich type apparatus	29 $\pm$ 2	29 $\pm$ 2

Chromatography of a mixture of Neher's dyes in a solvent mixture C_6H_6 -MeOH(9:1) is shown below. In this experiment, the same lot of silica gel (Wakogel B) was used for preparation of the thin-layer with a thickness of 250μ , and the development was carried out 10 cm. from the lower end of the plate.

Making use of the present apparatus, samples applied are chromatographed to give smaller and less diffused spots compared with the use of usual sandwich type apparatus.

Chromatographic mobilities ($R_f \times 100$) and their standard deviation ($SD \times 100$) of the dyes, obtained with 10 experiments, are shown in the following table. The data listed indicate that the present invention is superior in the reproducibility of the results.

Neher's dyes F-52, -59, -63, -11, -62, -22, -9, -14, -5

The apparatus in Fig. 1

$R_f \times 100$ 99, 79, 68, 53, 45, 37, 41, 34, 28

$SD \times 100$ 1, 1, 1, 1, 1, 1, 1, 1, 1

The apparatus in Fig. 4

$R_f \times 100$ 99, 80, 69, 53, 40, 36, 40, 33, 29

$SD \times 100$ 1, 2, 1, 1, 1, 1, 1, 1, 1

The usual sandwich type apparatus

$R_f \times 100$ 99, 60, 55, 46, 38, 36, 40, 37, 36

$SD \times 100$ 1, 3, 2, 3, 4, 2, 1, 2, 1

b. Solvent mixtures containing polar solvents cause little solvent demixing.

The previously proposed apparatus, such as the usual sandwich type apparatus, and the others designed by Brenner and the other workers (M. Brenner: Thin-layer Chromatography, A Laboratory Hand Book (E. Stahl ed.) p. 114, Academic Press 1965.) give a secondary solvent front (β -front) in every solvent system as far as examined and shown below, where the solvent front (α -front) is 1.

C_6H_6 -Me ₂ CO(4:1)	0.70-0.83
C_6H_6 -MeOH(9:1)	0.20-0.33
$CHCl_3$ -Me ₂ CO(3:1)	0.86-0.92
Hexane-EtOAc(2:8)	0.96-0.98
$CHCl_3$ -MeOH(97:3)	0.31
$CHCl_3$ -HOAc(3:1)	0.75

Not only is the β -front not observed with the present apparatus which have a porous medium inside the cover plate, but also they give much better separability of the sample components. This is based on an adsorption effect of the vapors of polar solvents which comes from the porous medium attached inside the cover plate.

What is claimed is:

1. An apparatus for effecting thin-layer chromatography which comprises:

- a coated support plate, wherein said coating comprises a thin-layer of adsorbent material;
- a cover plate for said support plate having a comparable width, wherein said cover plate has a porous medium disposed on the surface adjacent to the thin-layer of the coated support plate;
- a means for maintaining said cover plate parallel to and apart from said coated support plate and
- a solvent container in which the bottom edge of said support plate and said cover plate is immersed and exposed to solvent.

2. The apparatus of claim 1, wherein the means for maintaining said cover plate parallel to and apart from said coated support plate comprises spacers placed between and extending the length of the edges of said plates so as to minimize solvent vapor loss.

3. The apparatus of claim 2, wherein said means comprise spacers having a thickness of from about 1 to about 5 millimeters.

4. The apparatus of claim 2, wherein said means comprise spacers having a thickness of from about 2 to about 3 millimeters.

5. The apparatus of claim 1, wherein the porous medium disposed on the surface of said cover plate is selected from the group consisting of filter paper, glass fiber and fiber sheet.

6. The apparatus of claim 5, wherein said porous medium is impregnated with solvent prior to use.

7. The apparatus of claim 5, wherein said porous medium is retained adjacent to said cover plate by means of slender springs.

8. The apparatus of claim 1, wherein a movable heating means is disposed on the remote surface of said cover plate.

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