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Kremer

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[54] **CHROMATOGRAPHIC APPARATUS**

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[73] Assignee: **Schleicher & Schuell, Incorporated,** Keene, N.H.

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[52] **U.S. Cl.** ..... **210/198 C**

[51] **Int. Cl.²** **B01D 15/08**

[58] **Field of Search** ..... 210/31 C, 198 C

[56] **References Cited**

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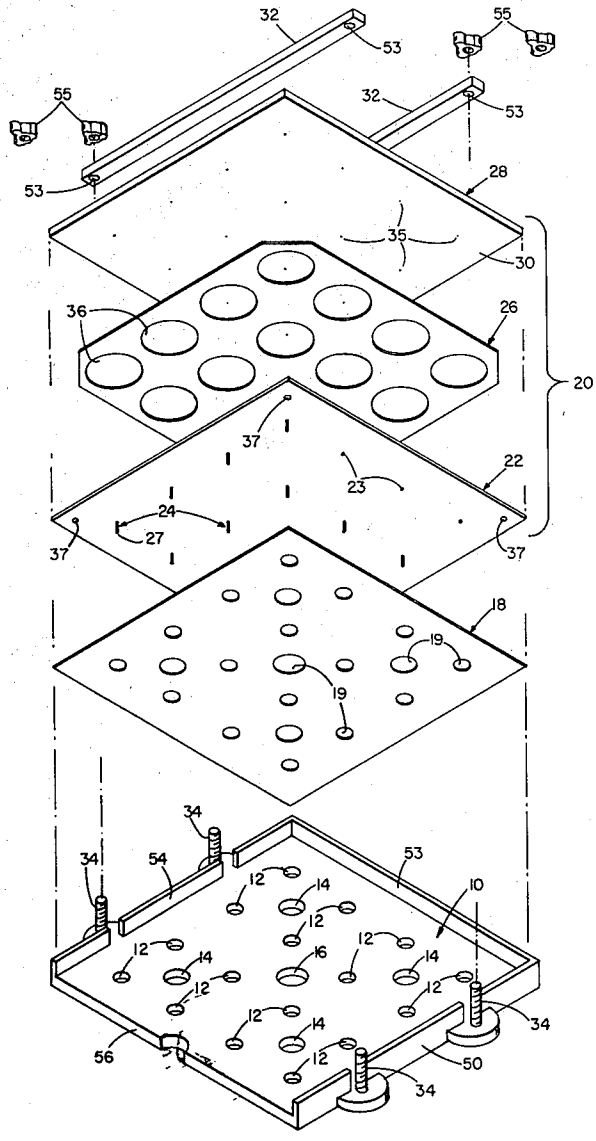
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Primary Examiner—John Adee

[57] **ABSTRACT**

Chromatographic apparatus for developing circular chromatograms on the sample spotted stationary layer of a TLC plate including: a base providing a pattern of solvent wells corresponding to sample positions facing the TLC plate, a wick holding plate between the base and the TLC plate, a sealing layer between the base and the wick holding plate with an aperture registering with each solvent well, wicks extending through the wick holding plate each with one end dipping in a well and the other end contacting a sample on the adsorbent surface, a spacing and sealing layer between the wick holder and the TLC plate with an aperture whose periphery surrounds each operative wick to provide a development chamber bounded by portions of each plate and such periphery, and means for clamping the assembly together to seal the chambers and assure contact of the wicks with the samples so that they will transport solvents from the wells to the samples.

6 Claims, 5 Drawing Figures



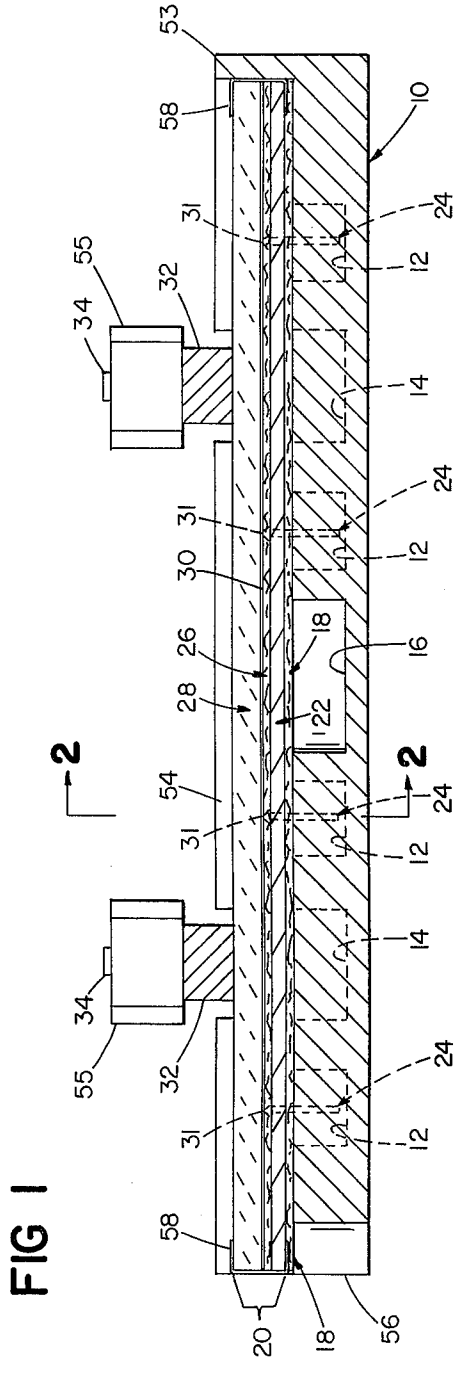

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FIG 1

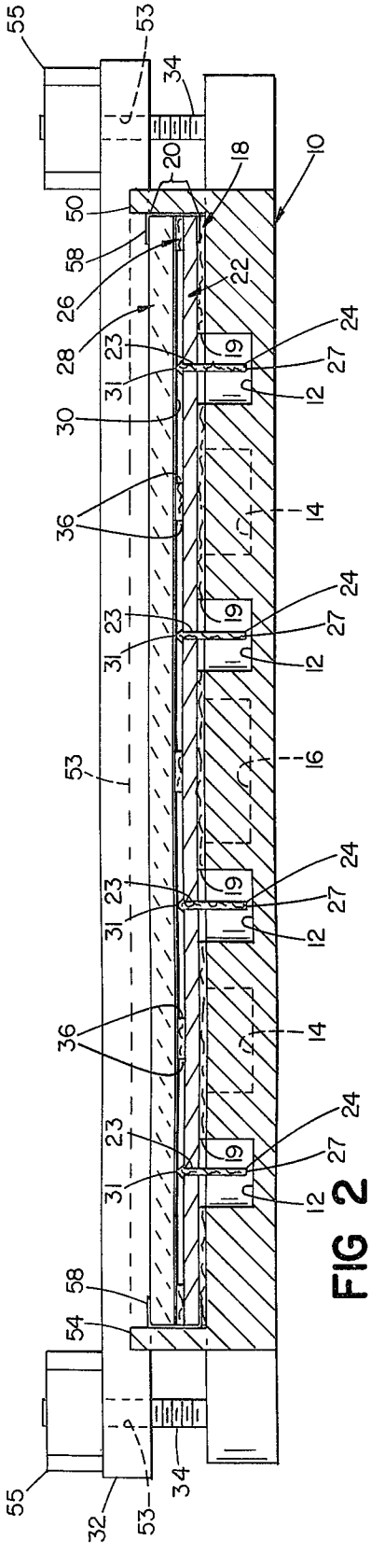
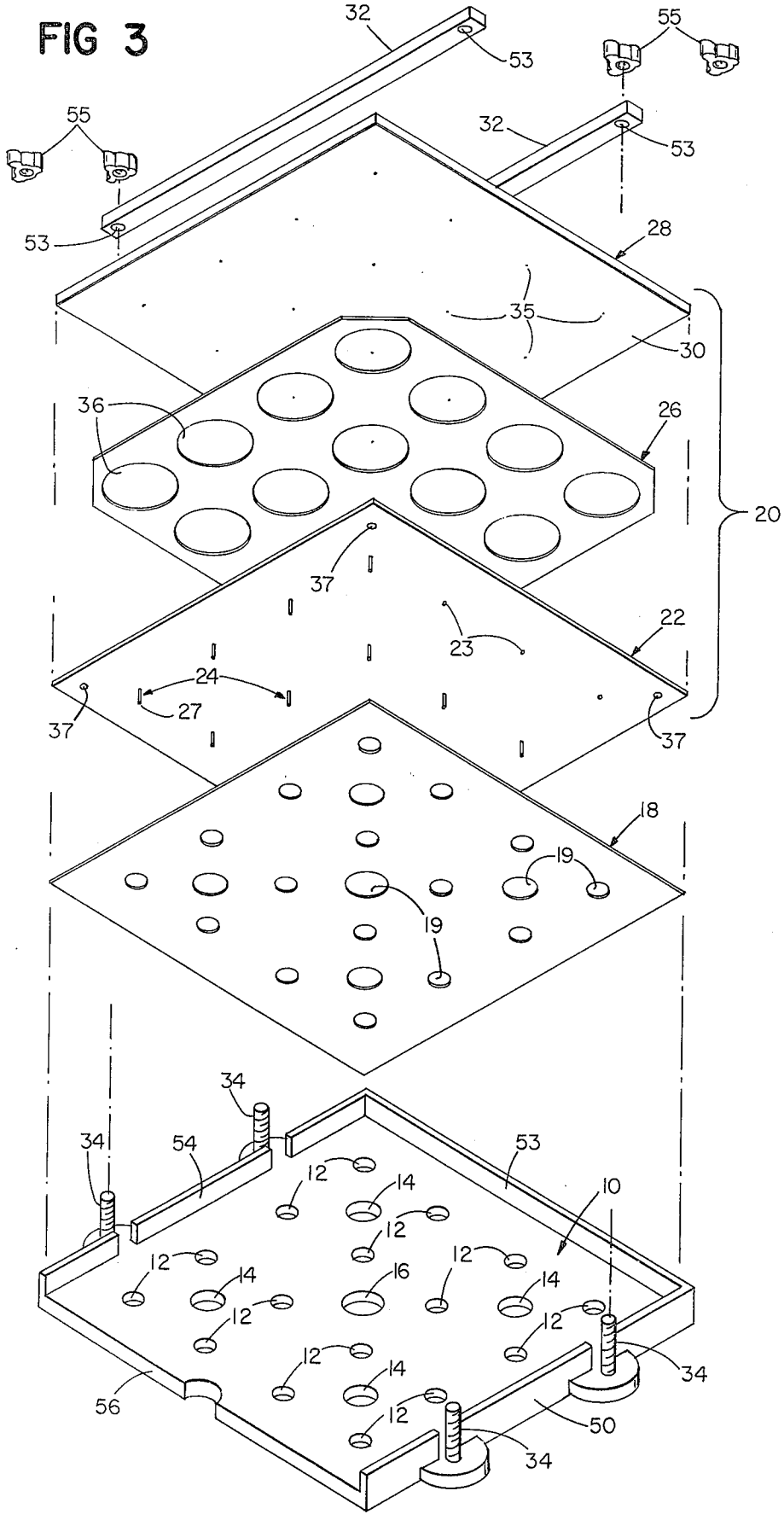

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FIG 2

FIG 3


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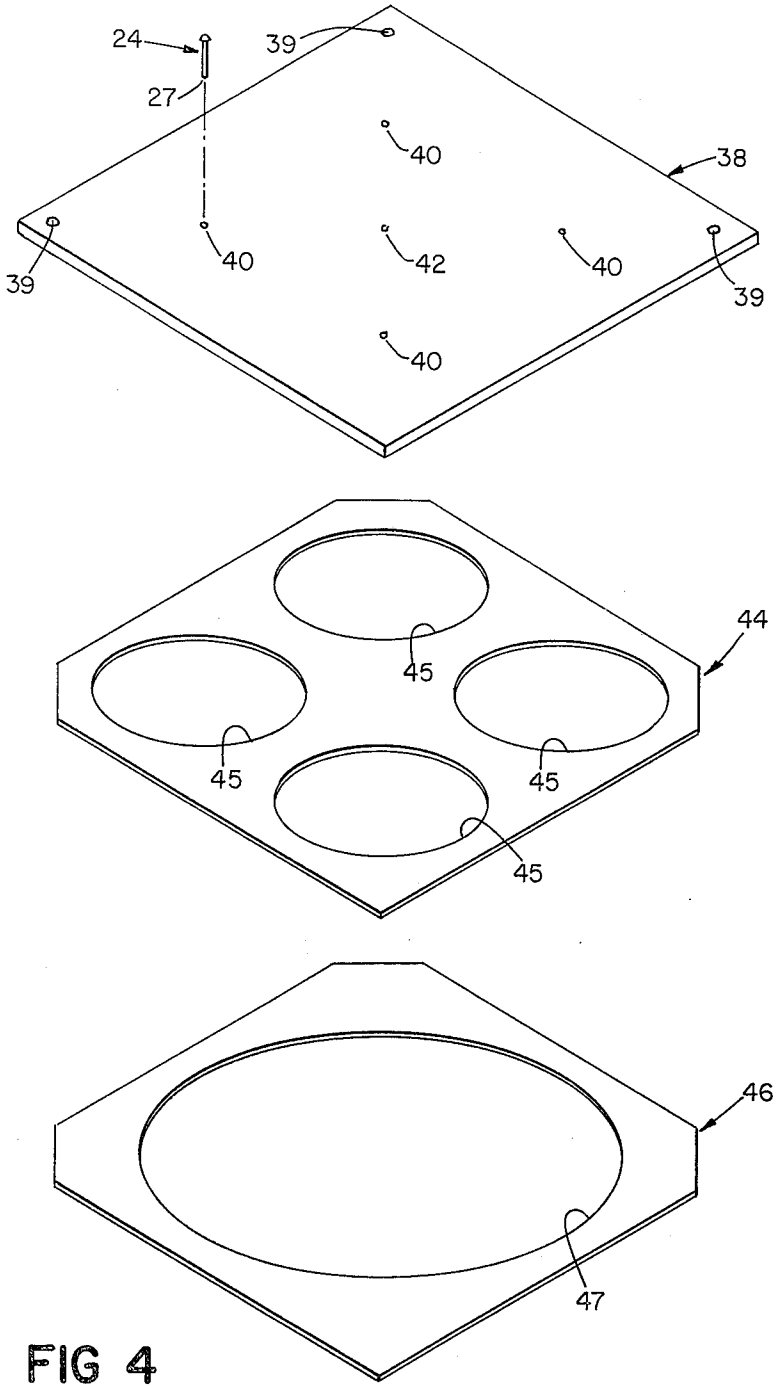


FIG 4

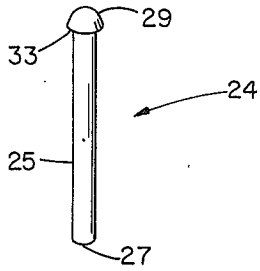


FIG 5

CHROMATOGRAPHIC APPARATUS

BACKGROUND OF THE INVENTION

This invention relates to circular thin-layer chromatography.

The initial major problem facing any chromatographer (whether in thin-layer chromatography or not), when asked to separate and analyze an unknown mixture or a previously unencountered mixture of knowns, or to optimize an existing scheme of separation and analysis, is the proper selection of the stationary and mobile phases (solvents) to be used in the chromatographic procedure. In their initial determination of the appropriate phases to be used, many chromatographers employ hand or commercially coated microscope slides, containing various stationary phases, comprising various solid adsorbents or liquid partitioning or reverse phase partitioning agents. Each of these slides in turn is placed in a small individual chamber containing a particular mobile phase, usually chosen from one of several suggested eluotropic series (series of solvents listed in the order of their increasing elutive power). An example of such a series is:

1. — Petroleum ether
2. — Cyclohexane
3. — Carbon tetrachloride
4. — Trichloroethylene
5. — Toluene
6. — Benzene
7. — Methylene dichloride
8. — Chloroform
9. — Diethyl ether
10. — Ethyl acetate
11. — Pyridine
12. — Acetone
13. — n-Propanol
14. — Ethanol
15. — Methanol
16. — Water

Based on the results of development in several such chambers, appropriate mobile phases (solvents) are selected for further work.

Circular chromatography is a particularly useful mobile phase selection tool because of its superior resolving power within a small development distance and time span.

Stahl has shown (Chemiken-Ztg. 82, 323 (1958), Parfumerie U. Kosm. 39, 564 (1958)) that the microcircular technique is excellently suited for quick determination of the requisite solvent. On pages 73 and 74 of his text Thin Layer Chromatography, 2d Ed, published by Springer-Verlag New York Inc., 1969, Stahl describes three devices for carrying out this technique: the standing pipette and two simple circular chambers.

However, as the system employing a standing pipette is open, it has the disadvantage that the solvent rapidly evaporates, and the chromatograms produced are therefore quite small, 1 to 2 cm in diameter. Another disadvantage of the system is that it is doubtful if the solvent ratio integrity of a multi-component solvent system is maintained, particularly if the component solvents differ appreciably in their volatility. It is also doubtful whether the chromatogram obtained is characteristic of what could be expected in a closed system, with a solvent saturated atmosphere.

The two circular chamber designs solve the problem of evaporation by utilizing a closed chamber system. But, as with the microscope slide technique mentioned above, only one solvent system can be evaluated by each chamber. This necessitates the use of a separate chamber for each solvent to be evaluated, and requires considerable time for preparation and clean up of such multiple apparatus. The developed chromatographs are all on separate plates and must be assembled and arranged systematically for purposes of comparison and evaluation. In addition, these chambers utilize a chromatographic plate which has been perforated from front to back to create a 2 mm diameter channel to accommodate a wick and/or allow solvent flow to the stationary layer on the plate.

SHORT STATEMENT OF THE INVENTION

An object of the present invention is to provide a chromatographic apparatus for the development of circular chromatograms that is simple in construction, compact, easy to use, and permits rapid evaluation of up to at least sixteen mobile phases (solvents) or samples, utilizing standard 20 × 20 cm chromatographic plates (TLC plates).

It is also an object to provide such a system that is economical in its use of solvent.

The apparatus of this invention does not require any subdivision of the stationary layer (i.e., adsorbent surface), as by scoring or other adsorbent removal, to create isolated channels or zones, as is normally done when different mobile phases are run simultaneously on a single plate.

In addition, the system of the invention may be quickly and easily set up for use, and, in particular, eliminates the time required to thread flexible wicking through wicking support means, which is a laborious and time-consuming operation.

The system of the invention also provides for simple selection of the number and size of developed chromatograms to be obtained.

With the foregoing in mind, the invention is featured by the provision of chromatographic apparatus for developing chromatograms on the stationary layer of a TLC plate spotted at predetermined sample positions with one or more samples, the apparatus including a base providing solvent wells in a predetermined pattern, each open at the upper surface of the base so as to face the stationary layer of the TLC plate when the latter is positioned in the apparatus. Selection means are provided adapted to be held between the base and the TLC plate to select solvent wells corresponding in number and position to the positions of the samples. The selection means comprises a wick holding plate arranged to be held between the base and the TLC plate, having holes corresponding both in number and position to the aforesaid sample positions and corresponding wells, the plate being adapted to hold wicks in said holes, each wick having a solvent take-up portion for dipping in a well and a sample contacting portion for contacting a sample; and a spacing and sealing layer between the wick holding plate and the TLC plate, such layer including a spacing aperture having a periphery surrounding each sample contacting portion and spaced therefrom to define a development chamber bounded by portions of the last named plates and the said periphery. Finally, pressure means are arranged to urge the plates together to seal each development chamber and to maintain the TLC plate in devel-

oping contact with each wick sample contacting portion, whereby each wick will transport solvent from its solvent well through its development chamber to a sample for development thereof.

A further feature is the provision of alternative selection means which can be substituted in the assembly for the selection means just described and comprising a second wick holding plate and a second spacing and sealing layer with a different number and arrangement of matching holes and apertures to correspond with samples spotted at other positions on the stationary layer of the TLC plate and their corresponding wells.

The base preferably is provided with three upstanding wall portions to receive and hold the plates and associated spacing and sealing felts in proper position and the pressure means preferably comprises upstanding studs mounted on the base and associated pressure bars and threaded knobs coacting with the studs to clamp the assembly together by means of the pressure bars.

A desirable further feature is the provision of a spacing and solvent isolating felt between the base and the wick holding plate provided with suitable apertures corresponding with the solvent wells in use so as to reduce evaporation and prevent spread of solvent vapor from one well area to another.

Further objects, features and advantages will become apparent from the following description of a preferred embodiment taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

In the drawings, illustrating a preferred embodiment of the invention,

FIG. **1** is a longitudinal cross-sectional view of the assembled apparatus preparatory to clamping the parts together;

FIG. **2** is a cross-sectional view taken on line **2—2** of FIG. **1**;

FIG. **3** is an exploded view in perspective showing the several components preparatory to assembly;

FIG. **4** is an exploded view of certain alternative components which may be used in the assembly to produce larger sized chromatograms; and

FIG. **5** is an elevation on an enlarged scale of a solvent transfer wick usable in the novel apparatus.

DESCRIPTION OF PREFERRED EMBODIMENT

Referring to the drawings, the components of a preferred embodiment of the chromatographic development apparatus of the invention are shown in the exploded views of FIGS. **3** and **4.** The system comprises a base plate **10** (FIG. **3**) providing three sets of solvent wells, all open at the upper surface of the base plate. There are 16 solvent wells **12,** each one-half inch (12.7 mm) in diameter, four solvent wells **14,** each three-quarters (19.04 mm) of an inch in diameter, and one central solvent well **16** that is 1 inch (25,38 mm) in diameter.

Base **10** further provides walls **50, 52** and **54** on three edges of the base, extending above the upper surface. The fourth edge **56** does not provide a wall but is open to permit other elements of the system to be slid into position and retained within walls **50, 52** and **54.** Exterior to each of the opposed walls **50** and **54** and attached to the base, by projections are two upstanding threaded studs **34** external to the base plate.

The chromatographic development system further comprises a base plate sealer felt **18** that covers the upper surface of base plate **10** and provides apertures **19** registering with wells **12, 14** and **16.** Felt **18** acts as a solvent isolating layer which reduces evaporation, and prevents mixing of the vapors from the solvents contained in the various wells.

Not all the solvent wells in base **10** are employed at the same time. The system of the invention makes possible the development of one chromatogram having a diameter of up to 170 mm, or in the alternative, from one to four chromatograms having diameters of up to 87 mm or, as a third alternative, from 1 to 16 chromatograms having diameters of up to 40 mm. The selection of one of these alternatives is made possible by the use of selection means, comprising one of the alternative wick holding plates combined with one of the alternative spacer felts, to be described.

As seen in FIG. **3,** one form of wick holding plate **22** provides 16 wick holes **23** corresponding with the 16 half-inch (12.7 mm) diameter solvent wells **12** in base plate **10.** 16 wicks **24** are inserted in wick holding plate **22** through holes **23.** In FIG. **3,** three of the wicks are not shown, in order to show the wick holes. In addition, plate **22** provides three registration holes **37,** placed asymmetrically with respect to the edges of the plate, whose function is explained below.

A wick (FIG. **5**) suitable for use in apparatus of the invention is molded of a porous solvent resistant wicking material, such as polyethylene or polypropylene. Other solvent resistant plastics, fibrous materials and ceramics could also be employed, but polyethylene and polypropylene are particularly suitable because they are commercially available, inexpensive, and provide firm body construction. The particular wick employed in the specific embodiment herein described is manufactured by Porex Materials Corp. for use in pens.

Each wick **24,** seen in detail in FIG. **5,** has an elongated cylindrical body portion **25** about 1/16 inch (1.85 mm) thick, including a solvent takeup base portion **27.** The wick further has a rounded head **29** about 1.50 mm long which acts as a sample contact portion and which includes a retaining edge **33** of larger diameter than body portion **25.** The wick is about ⅝ inch (18.0 mm) long overall.

A first alternative spacer and sealer felt **26** provides sixteen circular holes **36** in diameter 40 mm. The felt thickness is approximately equal to the length of wick head **29,** i.e., of the order of 2.60 mm. When the spacer felt is placed on wick holding plate **22,** the periphery of each spacer felt hole **36** surrounds one of the sixteen wick heads **29** protruding above plate **22.** A thin-layer chromatography (TLC) plate **28** provides a stationary layer **30** which has been sample spotted at locations indicated by reference numeral **35.**

The removable pressure bars **32** are provided with openings **53** spaced to receive the four upstanding studs **34** fixed to base **10.** Four threaded fastening knobs **55** are provided to engage the protruding ends of posts **34,** in order to press TLC plate **28** downwardly toward base **10,** to insure uniform good contact of wick points **31** against sample spots **35** and to insure sealing contact of the plates with the felts.

An alternative wick holding plate **38** (FIG. **4**) provides four wick holes **40** and one central wick hole **42.** The four wick holes **40** correspond with the 4¾ inch (19.04 mm) diameter wells **14** in base plate **10,** while the central wick hole **42** corresponds with the single

1-inch (25.38 mm) diameter well **16** in base plate **10.** In addition, plate **38** provides three registration holes **39,** asymmetrically placed with respect to the edges of the plate. Two further spacer felts **44** and **46** are provided for use alternatively with plate **38;** felt **44** provides four holes **45** each of diameter of 87 **mm, and felt** 46 **provides a single hole** 47 **having a diameter of** 170 mm.

When wick holding plate **22,** providing up to 16 wicks, is employed together with spacer felt **26,** any or all of the sixteen ½ (12.7 mm) solvent wells **12** in base plate **10** may be selected for use in developing as many as sixteen 40 mm diameter chromatograms on TLC plate **28.**

When wick holding plate **38** is employed with spacer felt **44,** with up to four wicks disposed in the four holes **40,** any or all of the four ¾ inch (19.04 mm) holes **14** in base plate **10** are selected, permitting development of as many as four chromatograms up to 87 mm in diameter. When wick holding plate **38** is employed with spacer felt **46,** with a single wick provided in central hole **42,** the central 1-inch (25.38 mm) well **16** in base plate **10** is selected permitting development of a single chromatogram up to 170 mm in diameter.

In use, the base plate sealer felt **18** is laid inside the side walls of the base plate **10,** so that the apertures **19** in the sealer felt and the solvent wells **12, 14** and **16** in the base plate are aligned with one another. A standard 20 × 20 cm TLC plate, such as plate **28,** is then laid on top of the sealer felt, stationary layer side **30** up. On top of this is placed a selected one of the two wick holding plates **22** and **38,** the choice being determined by the number and size of the developed chromatograms desired by the analyst. With a soft lead pencil, using the wick holes (**23, 40,** or **42**) and registration holes (**37** or **39**) as guides, the TLC plate is marked through the wick holes for sample spotting, and registration marks are made through the registration holes. The marked TLC plate and the wicking plate are then removed from the base plate and the desired samples are then spotted on the TLC plate at the marked locations.

Wicks **24** are then inserted into the appropriate holes in the selected wicking plate. The appropriate spacer felt (**26, 44** or **46**) is laid on top of the wicking plate and aligned therewith. Next, the sample spotted TLC plate **28** is laid on top of the spacer felt, this time with the stationary layer side **30** down, and aligned with the wick holding plate by means of the registration marks and the corresponding registration holes in the wick holding plate. The spotted samples are thereby positioned over the wicks. Once positioned, the wick holding plate, spacer felt and TLC plate are conveniently taped together as by tape **58** (FIGS. **1** and **2**) to form a layered sandwich (as indicated at **20,** in FIG. **3**), which may be easily handled without disturbing the alignment of the wicks and spotted samples. The wick holding plate and spacer felt serve as selection means to select the appropriate set of solvent wells.

A solvent application plate (not shown) having small apertures centered over the base plate wells, may be used during filling of the solvent wells, to reduce losses of volatile solvents by evaporation. The appropriate solvent wells in base plate **10** are filled by means of a dropper, micropipette, syringe, or other suitable means. Each ½-inch (12.7 mm) well **12** requires about 0.25 ml of solvent for development of a 40 mm diameter circle. Each ¾ inch (19.04 mm) well **14** requires about 1.25 ml of solvent for development of a 87 mm diameter circle. Combinations of solvent may be conveniently and easily made, using a dropper or pipette.

After the solvent wells have been filled, layered sandwich **20,** previously prepared as described, is aligned with and laid on top of the base plate sealer felt **18,** so that the takeup ends **27** of the wicks **24,** protruding below the wick holding plate, dip into the filled solvent wells. The pressure bars **32** are placed in position by sliding them down over the studs **34** until they are in contact with the glass (uncoated) upper surface of the TLC plate, which forms the top side of the layered sandwich. The pressure bars **34** are secured in place by means of threaded fastening knobs **55,** which are screwed down on the tops of the studs. The pressure thus exerted on TLC plate **28** by the bars assists in bringing all of the wick heads **29** into firm contact with the stationary layer **30** of the TLC plate. It also presses the selected spacer layer (**26, 44** or **46**) into firm contact with both the wicking plate and the TLC plate. Thus, a tightly sealed cyclindrical development chamber is formed by the wicking plate, the periphery of the spacer felt aperture, and the TLC plate around each sample spot with the wick head in contact therewith. Each such chamber is positioned above its individual solvent feed system and is isolated from all other such solvent feed systems. The atmosphere in each of these chambers will rapidly become saturated with the vapor of the particular solvent with which the wick is in contact. Thus equilibrium between the liquid and gaseous phases is quickly established.

The solvent is drawn up the wick by capillary action and comes into contact with the spotted sample on the stationary layer of the TLC plate, and chromatographic development begins. Development may be limited by limiting the quantity of solvent with which the well is initially filled, or the analyst may remove the sandwich package from the base plate in order to stop development when the solvent front reaches the edge of the hole in the spacer felt. The TLC plate is then separated from the sandwich package and dried, and thereafter is visualized in the conventional manner.

Using the set of sixteen ½-inch (12.7 mm) wells **12** in combination with selection means comprising spacer felt **26** and wick holding plate **22,** an entire eluotropic series can be run at one time. The series proposed by Stahl (Thin-Layer Chromatography, **2** Ed., Springer-Verlag New York Inc. 1969, p. 202) contains only 13 solvents; Truter (Thin Film Chromatography, Interscience, 1963, p. 29) lists **14.** Once the initial series has been run, a selected portion of a more extensive series can be used (Chromatography, **2** Ed., E. Heftmann, p. 59), or multiple systems can be tried, made up of those solvents which showed promise during the initial run. By using a single 20 × 20 cm plate in this manner, it is possible to eliminate the use of a larger number of individual tank type chambers, and to cut down on preparation time and cleanup time. Visualization of the chromatograms by ultraviolet light, reagent spraying, or the like is also facilitated because the chromatograms are all contained on one plate and can be visualized as a unit. Also, the finished chromatograms may be displayed compactly and in logical order, to facilitate comparison and evaluation of results.

When it is desired to develop larger size chromatograms on the same type of standard size TLC plate, alternative wick holder **38** may be employed and, with it, either of the two spacer felts **44** and **46.** Where four wicks are used up to four chromatograms up to 87 mm

in diameter may be developed; with a single central wick and felt **46,** a single chromatogram up to 170 mm in diameter may be developed.

While the invention has particular utility as applied to the utilization of standard sized TLC plates of 20 × 20 cm, it will be understood that the scope of the invention is not so limited and its advantages may be enjoyed using components of any suitable size.

What is claimed is:

1. Chromatographic apparatus for developing chromatograms on the stationary layer of a TLC plate spotted at predetermined sample positions with one or more samples, said apparatus comprising

a base providing solvent wells in a predetermined pattern, each open at the upper surface of the base so as to face the adsorbent surface of the TLC plate when the latter is positioned in the apparatus,

selection means adapted to be held between said base and said TLC plate to select solvent wells corresponding in number and position to the positions of said samples, said means comprising

a wick holding plate arranged to be held between said base and said TLC plate and having holes corresponding both in number and position to the aforesaid sample positions and corresponding wells,

said plate being adapted to hold wicks in said holes, each wick having a solvent take-up portion for dipping in a well and a sample contacting portion for contacting a sample, and

a spacing and sealing layer between said wick holding plate and said TLC plate, said layer including a spacing aperture having a periphery surrounding each said sample contacting portion and spaced therefrom to define a development chamber bounded by portions of said last named plates and said periphery, and

pressure means arranged to urge said plates together to seal each said chamber and to maintain said TLC plate in developing contact with each said wick sample contacting portion,

whereby each wick will transport solvent from its solvent well through its development chamber to a sample for development thereof.

2. The combination of claim **1** wherein said base is provided with wall portions extending above said base upper surface, for receiving and retaining said selection means and said TLC plate.

3. The combination of claim **1,** further including

a solvent isolating layer between said base upper surface and said wick holding plate, said isolating layer including an aperture registering with each solvent well opening.

4. The combination of claim **1,** wherein said pressure means comprises

a pair of studs extending upwardly on opposite sides of said base,

a removable pressure bar having mounting holes to receive said studs, and

threaded knobs engaging said studs.

5. A chromatography system comprising

a base including at least one solvent well open at the upper surface of said base

a wick holding apertured plate above said base

at least one wick extending from a solvent well through an aperture of said wick holding plate and extending thereabove

a spacing and sealing layer above said wick holding plate and having an aperture having a periphery surrounding each wick

a TLC plate above said spacing layer and having a stationary layer adjacent said spacing layer, said TLC plate being adapted to be spotted with a sample to register with each wick

said wick holding plate, said spacing aperture periphery and said TLC plate together defining a generally closed development chamber

whereby a solvent contained in said base solvent well is transported by said wick through said development chamber to said sample spotted TLC plate for development of said sample.

6. The combination of claim **1** wherein alternative selection means are provided to be substituted for the first named selection means, said alternative selection means comprising

a second wick holding plate and a second spacing and sealing layer having matching holes and apertures arranged in an different pattern from those of the first named selection means so as to correspond with other sample positions on said stationary layer and their corresponding solvent wells.

* * * * *