United States Patent [19]

Rosenthal

[11] **3,766,884**

[45] **Oct. 23, 1973**

[54] **APPARATUS FOR SAMPLE APPLICATION IN THIN LAYER AND PAPER CHROMATOGRAPHY**

[75] Inventor: **Arthur Rosenthal**, New Hyde Park, N.Y.

[73] Assignee: **The Long Island Jewish Medical Center**, New Hyde Park, N.Y.

[22] Filed: **June 28, 1971**

[21] Appl. No.: **157,117**

[52] **U.S. Cl.** ..... **118/58**, 23/253 R, 118/401

[51] **Int. Cl.** **B05c 11/00**

[58] **Field of Search** ..... 118/63, 58, 401, 118/506, 300; 73/61.1 C; 23/253 R; 33/34

[56] **References Cited**

UNITED STATES PATENTS

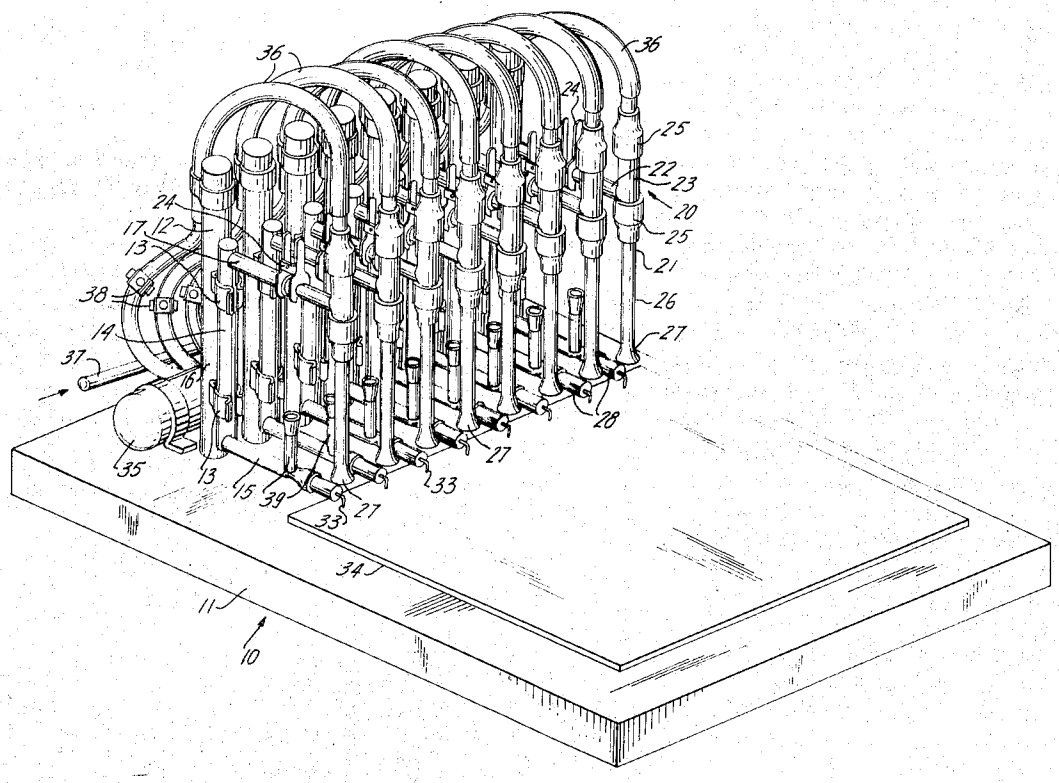
3,150,001	9/1964	Hrdina	118/58
3,136,609	6/1964	Ciagne	23/231 X
681,932	9/1901	Skirrow	33/34 UX

Primary Examiner—Morris Kaplan
Attorney—Sparrow & Sparrow

[57] **ABSTRACT**

An apparatus for producing spots of specimens of material on a plate having a thin layer adsorbing surface, the specimens being dissolved in a diluting solvent for chromatographic analysis, with a plurality of units, each one of the units comprising a glass tube which is filled with a specimen through a funnel-like glass filling tube projecting perpendicularly from the upper surface of the specimen tube, the latter also having an open front and which is stoppered or otherwise covered by a teflon or other inert plug through which passes a capillary tube, the capillary tube having a bent front end protruding from the open front end of the glass tube so that the end of the capillary is very close to the adsorbing surface of the plate, and an air tube above the front end of the capillary which may be adjustably set to blow a low-pressure air stream onto the specimen emanating from the capillary to evaporate the solvent, thereby forming a spot of the specimen on the thin-layer adsorbing surface of the plate.

24 Claims, 6 Drawing Figures

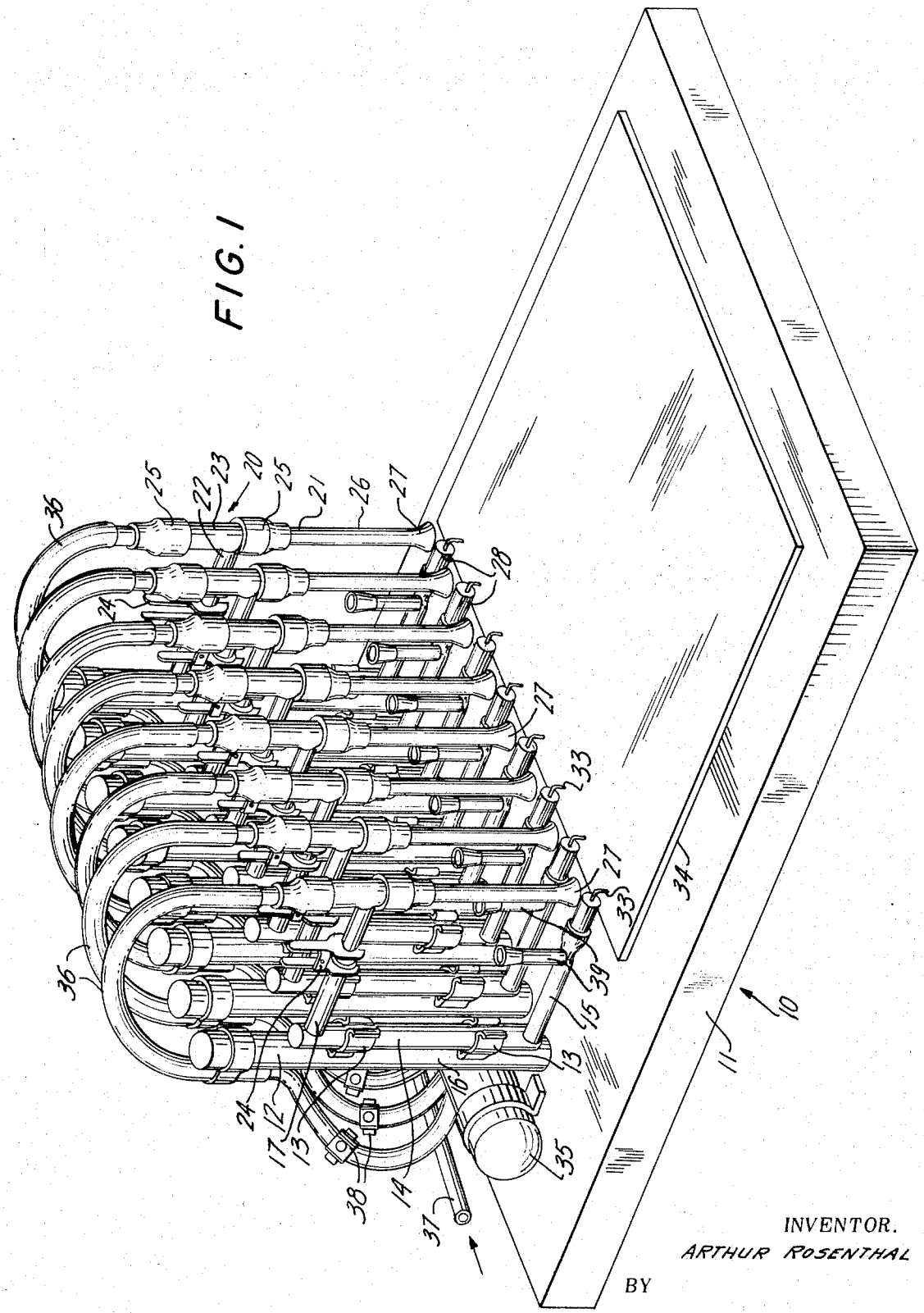


PATENTED OCT 23 1973

3,766,884

SHEET 1 OF 2

FIG. 1

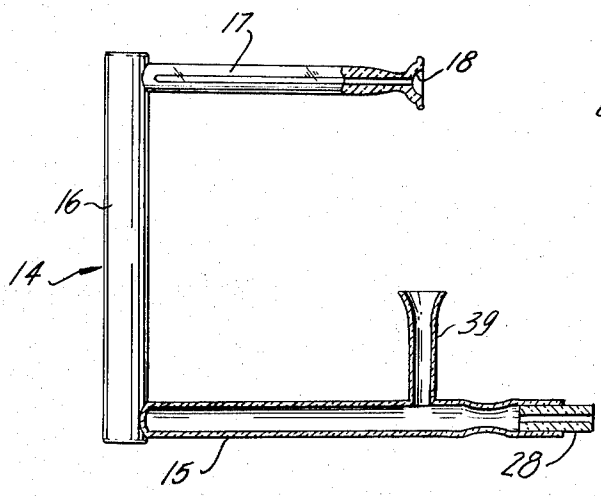


INVENTOR.
ARTHUR ROSENTHAL

BY

SPARROW AND SPARROW
ATTORNEYS

FIG. 2



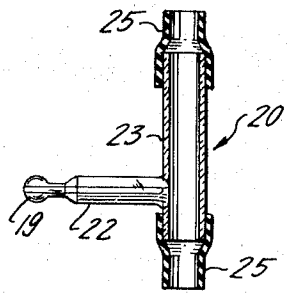


FIG. 3

FIG. 4

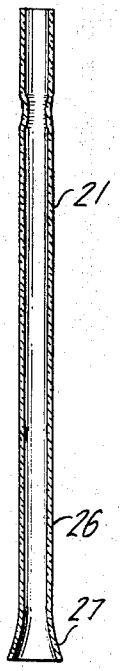


FIG. 5

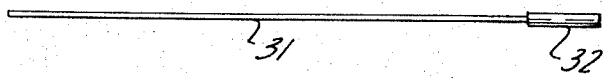
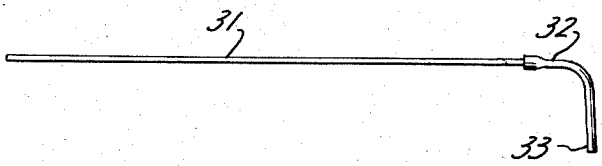


FIG. 6



INVENTOR.
ARTHUR ROSENTHAL

BY

SPARROW AND SPARROW
ATTORNEYS

APPARATUS FOR SAMPLE APPLICATION IN THIN LAYER AND PAPER CHROMATOGRAPHY

BACKGROUND OF THE INVENTION

The invention relates generally to medico-mechanical apparatus and particularly to apparatus for simultaneously preparing a plurality of specimens, especially for chromatographic analysis.

Various devices for producing spots for chromatographic analysis are known, some being built on an experimental laboratory basis in universities, some others being commercially produced. The known devices may be divided into the two main groups, one to produce specimens in streak form and the other to form individual "spots." The objective in making streaks is usually purification of a mixture for preparative purposes, while spot-making devices (as the presently described one) are used to facilitate qualitative or quantitative analysis of the chromatographically-separated materials. All prior art devices have several features in common. Either they are hand-operated, which is slow, tedious work, tying up laboratory personnel for the preparation of the specimens and delaying the chromatographic analysis, or else they employ devices such as bellows, pumps, or other motorized devices which make them bulky and/or expensive and may expose the sample solutions to contamination from pump tubing. The non-motorized devices apply liquid to the spots discretely rather than continuously; since the samples to be analyzed are diluted by a solvent, it is obvious that the solvent of each one of these spots has to be evaporated before another portion of solution can be applied to the spot, until a comlete spot is formed which contains a sufficient quantity of material for the chormatographic analysis. Another common feature found in the prior art is having either the base plate on which the spots are to be formed, or the pipet or capillary, move to obtain more than one spot or streak. Various operations are known to fill the pipettes or capillaries, all of which are cumbersome, expensive and/or time-consuming. All known devices have been built to be operated by skilled personnel who have to devote their full attention to the process of preparing the spots, and since this operation is strictly under manual control, it is more likely than not that the sizes and thicknesses of the spots obtained, as well as their positions relative to one another on the adsorbent base, will not be uniform and consistent.

The problems of time- and labor-saving production of the spots and of a more predictable and reliable consistency of quality of these spots, are intended to be solved by this invention.

SUMMARY OF THE INVENTION

The invention consists in such novel features, construction arrangements, combinations of parts and improvements as may be shown and described in connection with the apparatus herein disclosed by way of example only and as illustrative of a preferred embodiment.

The technique of the thin-layer chromatography relies first of all upon the formation of small spots on a support plate which has a thin layer of an adsorbent material, e.g. alumina, silica-gel, cellulose, etc., in a very finely divided form adherent to the plate. The samples to be analyzed may be of chemical, biological, or biomedical origin. In the present invention the samples are dissolved by a solvent (e.g., chloroform) which has an adequate and desirable rate of evaporation. Specimens of the magnitude of 1–2 ml are taken and deposited in the form of spots on the adsorbent surface by a continuous process, during which the solvent is continuously evaporated. The final spot contains, therefore, the entire quantity of the solids of the sample and yet does not have a size larger than 0.5 to 0.75 cm. The invention thus serves simultaneously to concentrate the specimen and apply it as a compact spot to a thin-layer plate, in preparation for chromatographic separation and analysis. Small, easily refillable "reservoirs" for the specimens are provided and capillary tubes are used to take the solution out of the reservoirs for continuous deposit on the support plate on the same spot. A supply of air under low pressure, which may or may not be heated, is directed down by way of a nozzle on top of the discharge end of the capillary tube. This flow of low-pressure air envelops the end of the capillary without causing an air turbulence, evaporating at the same time the solvent and holding the forming spot to its desired diameter. This feature enables the arrangement of a plurality of capillaries side-by-side, which can thus be used simultaneously. Since the support plate with the adsorbent surface layer has the standard width of 8 inches, the arrangement of 8 capillaries at distances of 1 inch from one another is easily feasible, without spots running into one another.

Objects and advantages of the invention will be set forth in part hereafter and in part will be obvious herefrom or may be learned by practicing the invention, the same being realized and attained by means of the instrumentalities and conbinations pointed out in the appended claims.

It is an object of the invention to provide a novel apparatus for simultaneously producing a plurality of spots of specimens for thin-layer chromatography and for paper chromatography.

Another object of the invention is to provide a new combination of parts for producing a thin-layer spot consisting of a sizable quantity of a sample under coninuous evaporation of the solvent of the sample.

A further object of the invention is to provide an apparatus by which reliably reproducible results can be obtained without continuous manual operation of the apparatus.

Furthermore, it is an object of the invention to provide an apparatus which can be operated expeditiously and without continuous supervision.

Yet another object of the invention is to provide an apparatus which is of simple structure and can be economically produced.

Various further and more specific purposes, features and advantages will clearly appear from the detailed description given below taken in connection with the accompanying drawing which forms part of this specification and illustrates merely by way of example one embodiment of the apparatus of the invention.

BRIEF DESCRIPTION OF THE DRAWING

In the following description and in the claims, parts will be identified by specific names for convenience, but such names are intended to be as generic in their application to similar parts as the art will permit. Like reference characters denote like parts in the figures of the drawing, in which

FIG. 1 is an isometric perspective view of the apparatus of the invention;

FIG. 2 is a part of the apparatus having the reservoir tube for the specimen and the support arm which the socket for the conventional ball-and-socket universal joint;

FIG. 3 shows the holder for the air tube, having the ball-shaped end constituting the counterpart for the socket shown in FIG. 2.

FIG. 4 shows the air tube with the flared nozzle end;

FIG. 5 is a plan view of the capillary tube which fits through the teflon plug which is seated firmly in the reservoir tube shown in FIG. 2; and

FIG. 6 is a side view of the capillary shown in FIG. 5.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Referring now in more detail to the drawing illustrating a preferred embodiment by which the invention may be realized, there is the complete apparatus of the invention shown in FIGS. 1, identified by the general numeral **10.** The component parts are mounted on a base **11,** preferably made of a corrosion-proof material. A plurality of vertical posts **12** (eight are shown in the Figure) are fixedly erected on base **11,** spaced apart in equal intervals in side-by-side relationship in a straight row. Conventional spring clips **13** are attached to posts **12** to hold the support parts **14** for the tube-like reservoirs **15** in place. Support parts **14,** which may be of glass, comprise an upright member **16,** the tube-like reservoir **15** affixed to the lower end of member **16** and inclined slightly upward, and a support arm **17** which is affixed to the upper end of member **16** at right angle thereto. The free end of support arm **17** is shaped as a socket **18** of a universal ball joint, into which the ball-shaped end **19** of the holder **20** for the air tube **21** fits. Ball-shaped end **19** is the end of a short arm **22** which is affixed to a tube **23** at right angle thereto. Ball end **19** is held in place in socket **18** by a conventional spherical-joint spring clamp **24.** Airtube **21** fits freely in glass tube **23** and is held in place therein by short rubber sleeves **25** in such manner that it is easily adjustable in axial direction. The lower end **26** of air tube **21** is flared to a bell **27.**

Tube-like reservoir **15** is slightly inclined from the horizontal and has a short stopper **28** plugging the front end thereof. The stopper **28** consists preferably of a solid piece of inert plastic (e.g. teflon or polyethylene) which securely covers the front end of reservoir **15,** but is removable. Capillary **31** passes through stopper **28** through an opening therein small enough to prevent lateral movement of capillary **31** in an air stream. This arrangement holds capillary **31** securely in place. A small funnel-like tube **39** projects from the upper surface of tube **15** just in front of its center through which reservoir **15** is filled without taking it out of the apparatus. Capillary **31** consists preferably of a piece of stainless steel hypodermic needle material. A short piece of an inert plastic capillary tube **32** is attached to the front end of capillary **31.** Plastic tube **32** is bent downward at right angle so that the end **33** thereof can be set very close to the adsorbent surface coating of the thin layer chromatographic plate **34** which is placed on base **11** underneath ends **33,** without the risk of damaging this coating. Conventional thin-layer plate **34** consists of a piece of thin glass or of a suitable plastic, the surface of which is very evenly coated with an adsorbent material such as silica gel, in which the line of spots of the specimens to be chromatographically anaylzed is formed. Behind the line of posts **12** is a manifold **35** mounted on base **11,** and air hoses **36** are attached to the upper ends of air tubes **21,** leading therefrom to manifold **35** which is, in turn, connected by a feeding air hose **37** to a conventional source of compressed air under low pressure. Laboratory-style Hoffman screw clamps **38** are arranged on air hoses **36** for a final adjustment of air supply to air tubes **21.** It is not absolutely necessary to heat the air, but a provision may be made to increase the temperature of the air above the ambient room temperature by conventional heating means (e.g. by encirling the glass manifold with a heating coil, by directing infrared light at the line of forming spots, or by having the base **11** be the top surface of a hot plate).

The apparatus is operated as follows:

The support parts **14** are vertically adjusted on posts **12** so that ends **33** of plastic capillaries **32** are very close to the coated surface of the chromatographic plate **34** which has been placed on base **11** so that the row of the ends **33** is approximately **1** inch away from the edge of the plate. Air tubes **21** are set vertically above the ends of the capillaries and are adjusted by the universal joint to a position where the flared nozzles **27** are above the ends of the capillaries. In the meantime, samples to be analyzed have been prepared by dissolving them in a solvent, for example, chloroform. Low pressure air is now turned on and the flow of air is individually adjusted by means of hose clamps **38.** Quantities of the diluted samples, generally **2** ml each, are filled through hole **39** into reservoir tubes **15.** Since tubes **15** are slightly inclined from the horizontal, the liquid will not flow onto the plastic stoppers **28,** but will pass slowly by the capillary action through capillaries **31,** and since ends **33** of the plastic capillaries **32** are slightly lower than the rear end of capillaries **31** in reservoir tubes **15,** a siphoning effect also takes place in feeding the liquid out of the reservoirs through the capillaries. The slow outflow of the samples will form relatively small spots as long as the air flowing out of flared nozzles will evaporate the solvent instantaneously, continuously preventing thereby a spreading of the liquid in the absorbent coating. The air nozzles are eventually adjusted to a desired position so that spots of approximately 0.5 to 0.75 cm diameter will result. The process continues fully automatically until the entire contents of the reservoirs are spent and the solids of the samples are accumulated in the spots. The reservoirs may be washed with fresh portions of solvent, if desired, and the wash solution added to the original spot by the same means used in the application of the latter. The plate with the spots thereon is now taken off the base and subjected to the conventional chromatographic analysis. The accuracy of the automated process is far greater and more consistent than one of a tedious manual operation, and better comparable analytic results can be obtained.

It is understood that the present invention may also be used similarly for the application of sample spots for paper chromatography.

While the invention has been described and illustrated with respect to a certain preferred example which gives satisfactory results, it will be understood by those skilled in the art after understanding the principle

of the invention, that various other changes and modifications may be made without departing from the spirit of the invention.

What is claimed is:

1. A spot applicator unit for thin layer and paper chromatography, which comprises

a. reservoir means for holding a measured amount of a liquid sample, said reservoir means having inlet means for filling said reservoir means and capillary tube outlet means for siphoning said measured amount of liquid sample out of said reservoir means, said capillary tube outlet means having a discharge end outside the reservoir means and below the level of a liquid sample in said reservoir means;

b. air supply means for flowing a stream of low pressure air to evaporate solvent from said liquid sample siphoned out of said reservoir means; and

c. support means for supporting said reservoir means and said air supply means with said air supply means arranged to flow air over a liquid sample discharged from said discharge end, said support means including adusting means for adjusting the position of said air supply means relative to said discharge end of said capillary outlet means thereby to control the rate of evaporation of solvent from said liquid sample siphoned out of said reservoir means.

2. The applicator unit according to claim 1, wherein said air supply means has a flared outlet end positioned adjacent said discharge end, the position of said flared outlet end being adjustable by said adjusting means.

3. The applicator unit according to claim 2, wherein said reservoir means has an elongated body portion, and said capillary outlet means comprises a capillary tube having a first part thereof inside said elongated body portion and in communication with a liquid sample in said elongated body portion, and said discharge end being provided by a second part of the capillary tube positioned outside of said body portion and arranged transverse to said elongated body portion.

4. The applicator unit according to claim 3, wherein said second part of said capillary tube is substantially perpendicular to said elongated body portion.

5. The applicator unit according to claim 3, wherein said first part of said capillary tube is of stainless steel and said second part is of an insert plastic secured to said first part.

6. The applicator unit according to claim 3, wherein said capillary tube is removably mounted in said elongated body portion.

7. The applicator unit according to claim 1, wherein said air supply means comprises air flow control means.

8. The applicator unit according to claim 1, wherein said air supply means comprises means for supplying heated air.

9. The applicator unit according to claim 1, wherein said capillary tube outlet means comprises a capillary tube having a first part inside the reservoir means and extending to the lowest part of said reservoir means and a second part outside the reservoir means and extending below the level of said lowest part of said reservoir means, said capillary tube outlet means being thus operable to siphon out the entire content of liquid sample in said reservoir.

10. Apparatus according to claim 1, wherein said capillary tube outlet means comprises a capillary tube having a first part inside the reservoir means and extending to the lowest part of said reservoir means and a second part outside the reservoir means and extending below the level of said lowest part of said reservoir means, said capillary tube outlet means being thus operable to siphon out the entire content of liquid sample in said reservoir.

11. Apparatus for analysis of multiple samples by thin layer and paper chromatography, which comprises A) a base for supporting a chromatographic paper or plate; B) a plurality of spot applicator units mounted in side-by-side relationship on said base, each spot applicator unit comprising

a. reservoir means for holding a measured amount of a liquid sample, said reservoir means having inlet means for filling said reservoir means and capillary tube outlet means for siphoning said measured amount of liquid sample out of said reservoir means and onto a paper or plate on said base, said capillary tube outlet means having a discharge end outside the reservoir means and below the level of a liquid sample in said reservoir means;

b. air supply means for flowing a stream of low pressure air to evaporate solvent from said liquid sample siphoned out of said reservoir means and onto a paper or plate on said base; and

c. support means for supporting said reservoir means and said air supply means with said air supply means arranged to flow air over a liquid sample discharged from said discharge end, said support means including adjusting means for adjusting the position of said air supply means relative to said discharge end of said capillary outlet means thereby to control the rate of evaporation of solvent from said liquid sample siphoned out of said reservoir means; and

C) the support means of said applicator units being mounted in spaced realtionship on said base.

12. Apparatus according to claim 11, wherein said air supply means has a portion extending substantially vertically, said vertical portion having a flared outlet end positioned adjacent said discharge end, the position of said flared outlet end being adjustable by said adjusting means.

13. Apparatus according to claim 12, wherein said reservoir means has an elongated body portion slightly inclined to the horizontal, and said capillary outlet means comprises a capillary tube having a first part thereof inside said elongated body pprtion and in communication with a liquid sample in said elongated body portion, and said discharge end being provided by a second part of the capillary tube positioned outside of said body portion and extending downwardly.

14. Apparatus according to claim 13, wherein said second part of said capillary tube is substantially perpendicular to said elongated body portion.

15. Apparatus according to claim 13, wherein said first part of said capillary tube is of stainless steel and said second part is of an inert plastic secured to said first part.

16. Apparatus according to claim 13, wherein said capillary tube is removably mounted in said elongated body portion.

17. Apparatus according to claim 11, wherein said posts are spaced apart from one another at regular intervals in a straight line.

18. Apparatus according to claim 11, wherein said adjusting means comprises a ball and socket universal joint.

19. Apparatus according to claim 11, wherein said air supply means comprises an air tube for each unit, an air manifold, flexible air hoses connecing said air tubes to said manifold, and air flow control means on said air hoses.

20. Apparatus according to claim 19, wherein said air flow control means consists of screw clamps.

21. Apparatus according to Claim 19, wherein said air supply means comprise means to heat said manifold.

22. Apparatus according to claim 11, including and means to heat said base.

23. Apparatus according to claim 11, wherein said capillary tube outlet means comprises a capillary tube having a first part inside the reservoir means and extending to the lowest part of said reservoir means and a second part outside the reservoir means and extending below the level of said lowest part of said reservoir means, said capillary tube outlet means being thus operable to siphon out the entire content of liquid sample in said reservoir.

* * * * *

UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,766,884 Dated October 23, 1973

Inventor(s) Arthur Rosenthal

It is certified that error appears in the above-identified patent and that said Letters Patent are hereby corrected as shown below:

Cancel claim 10 and insert the following:

10. The applicator unit according to claim 3, wherein said inlet means for filling said reservoir is provided by a vertical funnel-like tube projecting upwardly from said elongated body portion; and plug means are provided in one end of said elongated body portion, said capillary tube passing through said plug means.

24. Apparatus according to claim 13, wherein said inlet means for filling said reservoir is provided by a vertical funnel-like tube projecting upwardly from said elongated body portion; and plug means are provided in one end of said elongated body portion, said capillary tube passing through sai plug means.

Claim 17, line 2, "posts" should read -- support means --.

Signed and sealed this 17th day of September 1974.

(SEAL)
Attest:

McCOY M. GIBSON JR.
Attesting Officer

C. MARSHALL DANN
Commissioner of Patents

FORM PO-1050 (10-69)

USCOMM-DC 60376-P69
* U.S. GOVERNMENT PRINTING OFFICE : 1969 O—366-334.