

[54] **CHROMATOGRAPHIC MATERIAL**

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- [52] U.S. Cl. **210/198**
- [51] Int. Cl. **B01d 15/08**
- [58] Field of Search 210/31, 198

[56] **References Cited**

UNITED STATES PATENTS

- 3,553,067 1/1971 Dwyer et al. 210/198 X

Primary Examiner—J. L. DeCesare
Attorney—Eugene O. Retter and George W. Rauchfuss, Jr.

[57] **ABSTRACT**

A thin layer chromatographic strip in the form of an elongated tape carrying indicia related to chromatographic test areas, the areas being in contact with a reservoir for solvent, the combination of test areas, indicia, and reservoir being periodically reproduced on the length of the tape, whereby the indicia may be used to initiate sampling, solvent application, and control passage through development and drying stages, and initiation and termination of scanning stages, thereby to make possible a sequence of unrelated or related analyses automatically on said tape.

10 Claims, 5 Drawing Figures

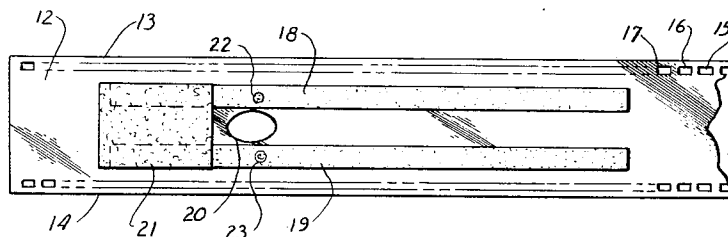


FIG. 1

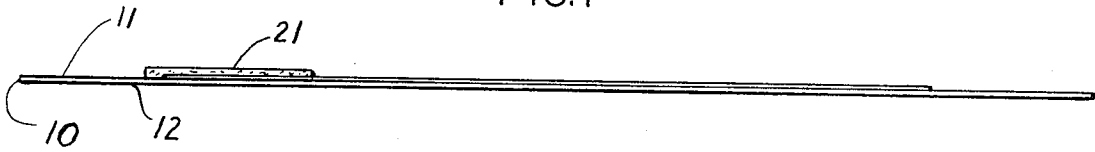


FIG. 2

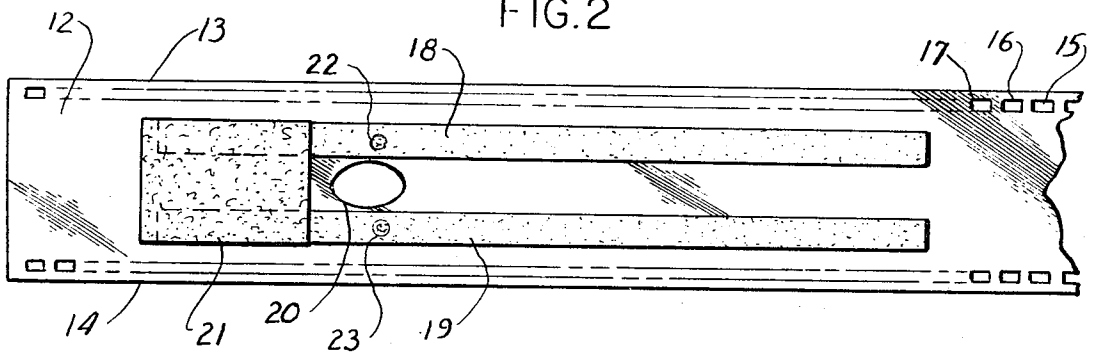


FIG. 3

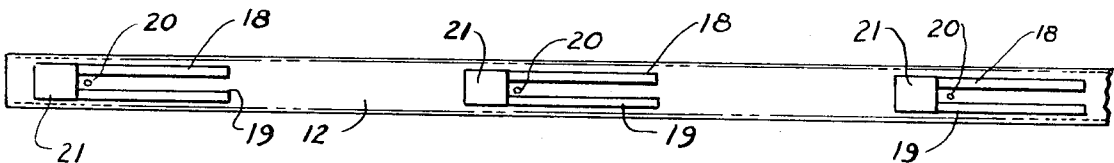


FIG. 5

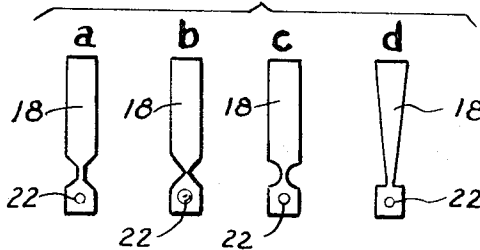
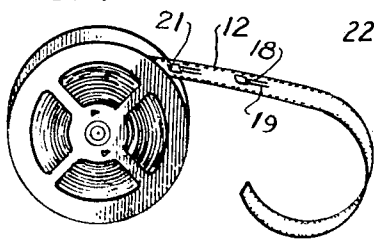


FIG. 4



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CHROMATOGRAPHIC MATERIAL

BACKGROUND OF THE INVENTION

The theory and practice of thin layer chromatography, column chromatography, and the theory of having a solvent move components of a mixture at different rates through an adsorptive medium is well documented. In thin layer chromatography, the fundamental technique is to apply a thin layer of adsorbent material to a substrate, usually a glass slide, or a large plastic sheet. The technique of preparing the thin layer involves the use of various mechanical coaters which will apply an appropriate layer of adsorptive material to this substrate, generally 50 microns to 300 microns. The material thus coated serves as an adsorption medium and the test technique involves placing a drop of the material to be separated at one point on the adsorptive surface, applying solvent continuously to the base of that adsorptive surface and allowing it to advance the spot by capillary action, with the entire test specimen being enclosed in an atmosphere of solvent vapor enclosed within a chamber. In the course of a few minutes the sample being separated is transported, through the adsorbent layer or alternately adsorbed and desorbed from the adsorbent, and its components separated into distinct areas depending upon the partition coefficient of each compound. The chromatogram then can be allowed to react with a chemical or with other mixtures of chemicals to produce color, if they have none, and scanned to relate intensity of color to the amount of material in the spot. Analysis becomes possible when the technique is carefully applied. The technique is of particular applicability in installations where many tests of a repetitive type are required, for example, the testing of body fluids in hospital laboratories. It is also in wide use in biomedical laboratories as a standard research tool.

It is, accordingly, a fundamental object of this invention to provide a chromatographic adsorptive test material formed on a flexible back material in the form of a continuous strip or tape to permit a continuous moving analysis of a large number of specimens from related, identical, or diverse sources, the tape carrying indicia rendering it possible to relate specimens to test results, through use of an automatic thin layer chromatography instrument.

Other objects and advantages of the invention will in part be apparent from the following description and drawings and in part appear hereinafter.

DETAILED DESCRIPTION OF THE INVENTION

This invention, is, accordingly, embodied in a tape, for example, a plastic tape as a base, the tape being characterized by its having mechanical indicia along its edges to permit precise timed movement of the tape over a timing sprocket, the tap having mounted on a face thereof a strip or strips of chromatographic adsorbent of predetermined width, length, and shape, coordinated with a reservoir for holding a chromatographic solvent, or solvent mixture, the tape being further coordinated with an indicium related to the placement of said reservoir and adsorptive surfaces, the several parts being coordinated so that in forward movement on a timed basis, a test specimen for chromatography can be applied to the surface of the adsorbent in response to one of said indicia, be dried, and travel through a development zone and drying zone, and also proceed in response to said indicia to a final stage of inspection, such as, for example, by visual or by optical means, for the identification of each of the zones in the chromatogram.

Thus, the invention broadly is embodied in the combination consisting of a base tape, in the form of reels of any size, which serves as a substrate and spaced strips of chromatographic adsorptive material thereon in spaced sequence, said strips individually being in contact with solvent reservoir pads. The adsorbent coating may be any material, with or without indicating dyes, such as silica gel, alumina, cellulose, derivatized cellulose, microcrystalline cellulose, kieselguhr, ion-exchange resins, polyamide powders, hydroxapatite, "Celite" diatomaceous earth, "Sephadex" polysaccharide dextran

derived powder, zinc carbonate, polyethylene powder, calcium sulfate, magnesium silicate, calcium hydroxide, "Florisil," magnesia-silica gel, and the like, and it is coated on the base in any manner such as by dropping, pouring, dipping, spraying or spreading, to form a thin layer on the substrate upon which mixtures of materials may be separated when the layer is dried.

The base, or tape, may be nylon, Dacron polyester, fiber made from polyethylene terephthalate or other monofilamentous fiber, the only requirement being that it be non-adsorptive and inert. It may vary in thickness, in fiber type, and class of weight. Metal foil is also useful as a substrate. The adsorbents may or may not require the use of a binder such as polyvinylpyrrolidone, polysaccharides, polyethers or calcium sulfate hemihydrate for extra adhesion. A preferred form of film base is the Mylar polyester tape commonly used in photography, and, in particular, in a specific form of our invention in Mylar, film, 35 millimeters in width, as used for motion picture film, the adsorptive strips, reservoirs and indicia being applied thereto.

The timing indicia used in the film may be the conventional perforations along the edges thereof, alone, or they may be an additional perforation located in appropriate relationship to the reservoir, or they may be in the form of conductive spots applied to the film in appropriate relation to the reservoir. The advantage of the perforation as an indicium is that in a traverse in an automatic apparatus the leading and lagging edges of the perforation are mechanically detectable with microswitches. The conductive spot is very useful, and can be sensed with electrical contacts and generally conventional microswitches. The magnetic spot is similarly readily detectable.

The construction and arrangement of the invention herein may be better understood by reference to the drawings and detailed description:

FIG. 1 is a side view exaggerated in thickness to show the relationship of chromatographic layer to the film;

FIG. 2 represents a plan view of a single chromatographic combination applied to a tape;

FIG. 3 is a general plan view of the length of tape showing a plurality of these chromatographic test strips mounted thereon;

FIG. 4 is a perspective view of a reel of tape showing a sequence of the chromatographic strips;

FIG. 5, *a, b, c, d* indicates the applicability of shaped strips to the invention.

Referring to FIG. 1, 10 represents a base of monofilamentous material or tape having an upper face, 11, a lower face, 12 and edges 13 and 14. Near each edge are perforations in precisely spaced sequence, namely, perforations 15, 16, 17, etc.

In the clear area between the rows of perforations 15 and 16 are mounted the chromatographic adsorptive areas 18 and 19 which are of a pre-selected length, carefully placed on the strip in relation to indicium 20. A preferred form of indicium is a perforation a few millimeters in width or diameter. Also included in specific relation to indicium 20 is the solvent reservoir or pad 21.

The combination of adsorptive area, indicium, and solvent reservoir is repeated at spaced intervals, as frequently as the length of tape being used will permit or less frequently as desired. The tape may further provide at each end a lead area for the indication thereon of data concerning the date, time, operator, chromatographic conditions and nature of the test. By relating the indicium to the test area the application of sample to adsorptive area and solvent to the reservoir can be coordinated with movement of the tape through test apparatus.

It is apparent that while the invention is illustrated in terms of two parallel test areas each about 5 to 8 millimeters in width, and as much as 80 to 100 millimeters in length, that the test area could be a single area approximately 20 millimeters in width or it could be made 3 or 4 chromatographic areas or

the like. For general use, it has been found that the parallel pair of areas represents a preferred form. They permit analysis of an unknown sample in one area and a known sample in the adjacent area for comparison. Wedge shaped areas as indicated in FIGS. 5, a, b, c, d are useful.

In use the tape can be manually or automatically driven through an analyzing machine which will automatically cause a specimen to be applied to each test area, and also initiate the application of solvent to the reservoir pad. In traversing through the development chamber of test apparatus which is saturated with solvent vapor, the solvent is given time to pass completely through the adsorbent layer and thereby effect separation of a mixture of compounds.

The chromatographic strips 18 and 19, may be of any of a variety of compositions suitable for performing chromatographic separations, or any of a variety of shapes as indicated in FIG. 5. It is understood that the use of the appropriate adsorbent will depend upon the nature of the compounds which will be separated. Necessary to the development of the chromatogram is a solvent, or mixture of solvents, which will perform the separation in combination with the absorbent coating in each strip. The reservoir pad 21 has been designated as the material onto which the solvent will be deposited. As indicated, the reservoir pad is squared off and thus provides a base line from which the solvent proceeds to migrate along the chromatographic strip. The material of which the reservoir is made and the adhesive holding it in place should be inert to the particular solvents to be used. Generally, it has been found the polyurethane foams or sponges are particularly useful as reservoir pad material. As solvent pads or reservoirs any material having an open cellular structure, e.g., cellulose, felt, cloth, inert to the solvents and capable of retaining solvent in the amount of 2 1/2 times the volume of the adsorbent layer is useful. They are simply lightly glued in direct contact with the base of each of the chromatographic adsorption strips. Thus, in developing a chromatogram, solvent is applied to the sponge 21 which serves as a solvent reservoir, in contact with the strips 18 and 19 and provides an accurately-defined base line, and solvent can then migrate along the strips 18 and 19 at a rate determined by its physical properties and the properties of particles making up the strips and by the solvent polarity. It contacts the origin of the chromatogram at positions 22 and 23 and in the course of a few minutes migrates through most of the length of 18 and 19 in order to complete the separation of the mixture of compounds originally deposited at the origin.

Virtually any of the various adsorbents which have been mentioned can be slurried and placed on the monofilament cloth or plastic tape as is done routinely in the usual thin layer preparation methods. Depending upon the adsorbent which is placed on the tape alkaloids, amines, amino acids, peptides, proteins, steroids, lipids, carotenoids, chlorophylls, vitamins, quinones, drugs, poisons, antibiotics, phenols, nucleic acids, nucleotide coenzymes, sugars, insecticides, dyes, miscellaneous organic compounds, inorganic ions, and other compounds in need of separation can be separated with this technique.

Specifically, in practicing the invention any of a variety of combinations of substrate with adsorbent can be used, but the following should be considered illustrative:

EXAMPLE I

A monofilament nylon strip, 25 millimeters wide, 0.1 millimeter thick, was coated in pairs of strips with a silica gel slurry, in sequence as shown in FIG. 2, containing a calcium sulfate binder. The strip was hung up to dry. In 15 minutes the strip was completely dry. The coated tape was hung in an oven at 110° C. for 10 minutes and removed, allowed to cool slightly in the air, and was spotted with 4 microliters of Stahl dye mixture. Solvent was applied to sponge 21 and the strip was then placed in a large volume tank saturated with solvent vapor and was developed to 8 centimeters from the origin. The separation of the three dyes was complete and took 6 minutes, the butter yellow, indophenol blue, and Sudan Red G formed distinct spots, well separated.

Depending upon the thickness of the slurry used to coat these nylon strips, the time for development will vary. The thicker the layer on the strip, the longer will be the development time.

EXAMPLE II

A length of monofilament cloth 25 millimeters wide, 100 centimeters long, and 0.1 millimeter thick was pulled through a silica gel slurry, as in Example I, and was wiped off with a doctor blade to remove excess slurry. With this method, a continuous strip of evenly coated monofilament nylon was made. This strip was air-dried and then cut into sections corresponding to 18 and 19 of FIG. 2 for individual chromatographic separations. The sections are glued on tape. A sponge reservoir then is glued in contact therewith, solvent is applied to the sponge and the operation completed as described in connection with Example I.

EXAMPLE III

A 35 millimeter Mylar type, 0.1 millimeter thick, was masked to leave parallel spaced strips uncovered, and sprayed with a silica gel slurry containing 1 percent NP-90 Polyvinylpyrrolidone as binder. The tape was allowed to air dry for 30 minutes, and sponges were applied as indicated in FIG. 2. The coated strips were activated for 10 minutes at 110° C. The coated strips were then spotted with 4 microliters of 0.1 molar solutions of o-nitroaniline, m-nitroaniline and p-nitroaniline in benzene. The coated strips were then passed in a chromatographic chamber saturated with solvent vapor, [phenol/water (8:3)], the sponge activated with 0.5 milliliter phenol/water (8:3) solvent and the strips developed to 10 centimeters from the origin. The 3 components separated completely, to give R_f values of 0.55, 0.44 and 0.37 respectively.

In recapitulation it is to be understood that the chromatographic adsorption device constituting this aspect of the invention consists of the flexible non-absorptive and non-adsorptive substrate serving as a carrier for adsorptive areas arranged to receive samples and to be in contact with a solvent reservoir, the combination being oriented in a fixed defined relationship with an indicium, preferably a perforation in the substrate, by arranging the combination in linear repetitious sequence along a tape. The several indicia can serve to provide together signals for sample application, solvent application, etc. and other intelligence built into a sequence where the operation involves a sequence of tests. The hole is incorporated in the tape to be used in combination with an automatic chromatographic apparatus, but it should be clear that the tape can be manipulated manually through an apparatus. A suitable apparatus for conducting tests using a substrate in chromatographic areas made in accordance with this invention is described in copending application filed of even date herewith, which application should be read as fully incorporated herein, Ser. No. 93,064, dated Nov. 27, 1970.

The adsorptive materials which may be used in the chromatographic layers are subject to virtually infinite variation and will vary depending upon the requirements of the tests to be conducted. For example, U.S. Pat. No. 3,418,152, W. S. Staudenmayer et al., issued Dec. 24, 1968, discloses a large group of specific exemplary formulations for laying down chromatographically active layers on a substrate and that disclosure may be considered fully incorporated herein. Similarly, U.S. Pat. No. 3,415,158, E. S. Perry et al., issued Dec. 24, 1968, also discloses a number of specific formulations and it may be considered fully incorporated herein.

I claim:

1. A thin layer chromatographic adsorption element in the form of an elongated strip suitable for use in sequential testing of specimens which comprises, in combination, an inert, non-adsorptive flexible substrate, a layer of chromatographic adsorbent material as a test area on a face of said substrate, a solvent reservoir on the face of said substrate and in abutting contact with the layer of adsorbent material, and indicia means along said substrate, said indicia means adapted to pro-

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vide signals to initiate sample application to the adsorbent material layer and solvent application to the solvent reservoir, said substrate including a repetitive sequence of said layers of chromatographic adsorbent materials, solvent reservoirs and indicia means spaced thereon.

2. A chromatographic adsorption element in accordance with claim 1 in which the substrate is a flexible plastic tape.

3. A chromatographic adsorption element in accordance with claim 1 in which the substrate is a monofilamentous fabric base.

4. A chromatographic adsorption element in accordance with claim 2 wherein a plurality of layers of chromatographic adsorbent material are arranged in parallel relationship on said flexible plastic tape substrate.

5. A chromatographic adsorption element in accordance with claim 1 wherein said layer of chromatographic adsorption material is selected from the group consisting of silica gel, alumina, cellulose, derivatized cellulose, microcrystalline cellulose, kieselguhr, ion-exchange resins, polyamide powders, hydroxapatite, diatomaceous earth, polysaccharide dextran derived powder, zinc carbonate, polyethylene powder, calci-

um sulfate, magnesium silicate, calcium hydroxide, and magnesia-silica gel.

6. A chromatographic adsorption element in accordance with claim 5 wherein said substrate is formed from a material selected from the group consisting of monofilamentous fabric of synthetic fiber, synthetic plastic, synthetic plastic-coated paper, synthetic plastic-coated fabric and metal foil.

7. A chromatographic adsorption element in accordance with claim 1 wherein the element is in the form of a reel of elongated tape having a sequence of chromatographic adsorptive material layers spaced along the length of the tape.

8. A chromatographic adsorption element of claim 2 wherein said indicia means comprises perforations spaced along the flexible plastic tape.

9. A chromatographic adsorption element in accordance with claim 2 wherein the indicia means comprises conductive spots spaced along the flexible tape.

10. A chromatographic adsorption element in accordance with claim 2 wherein the solvent reservoir comprises a pad of inert, open cellular structure material.

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