United States Patent

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[54] APPARATUS FOR CONTINUOUS LIQUID-SOLID PHASE CHROMATOGRAPHY

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[73] Assignee: **The United States of America as represented by the Secretary of Agriculture**

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

[51] **Int. Cl.** **B01d 15/08**

[58] **Field of Search** 210/31, 198; 55/67, 197, 386; 73/23.1

[56] **References Cited**

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[57] **ABSTRACT**

An apparatus for continuous liquid-solid phase chromatography having a column formed by two concentric cylinders clamped to a bottom plate which rests on a turntable operably connected to a drive means. A flat fixed phase bed surface for even layering of a solution and uniform entry of solution into the fixed phase is obtained by a unique accessory, a leveling plow.

1 Claim, 7 Drawing Figures

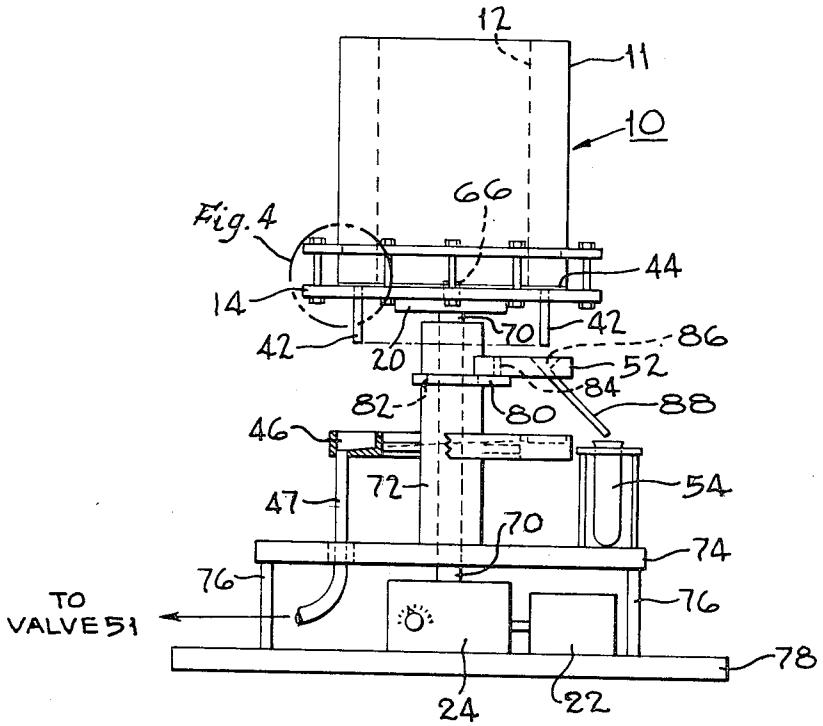


Fig. 1

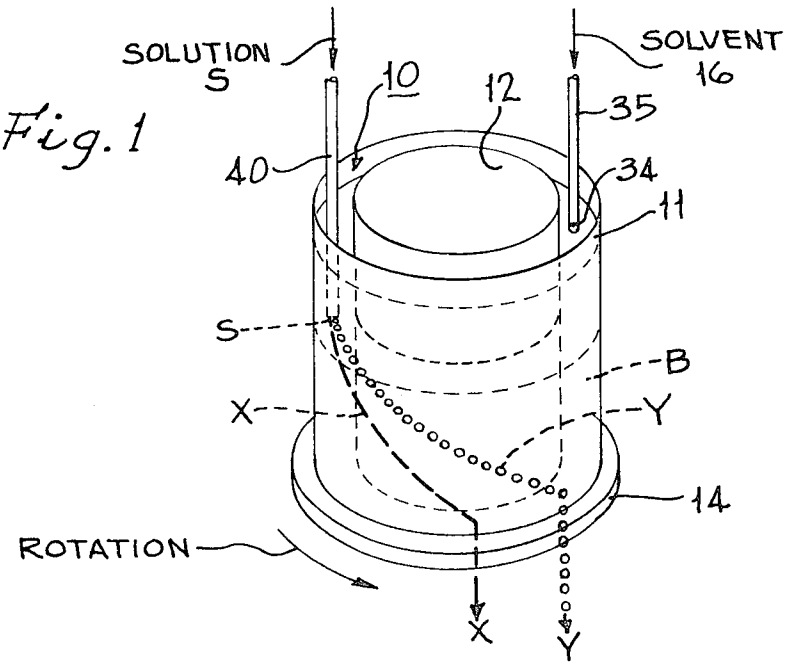
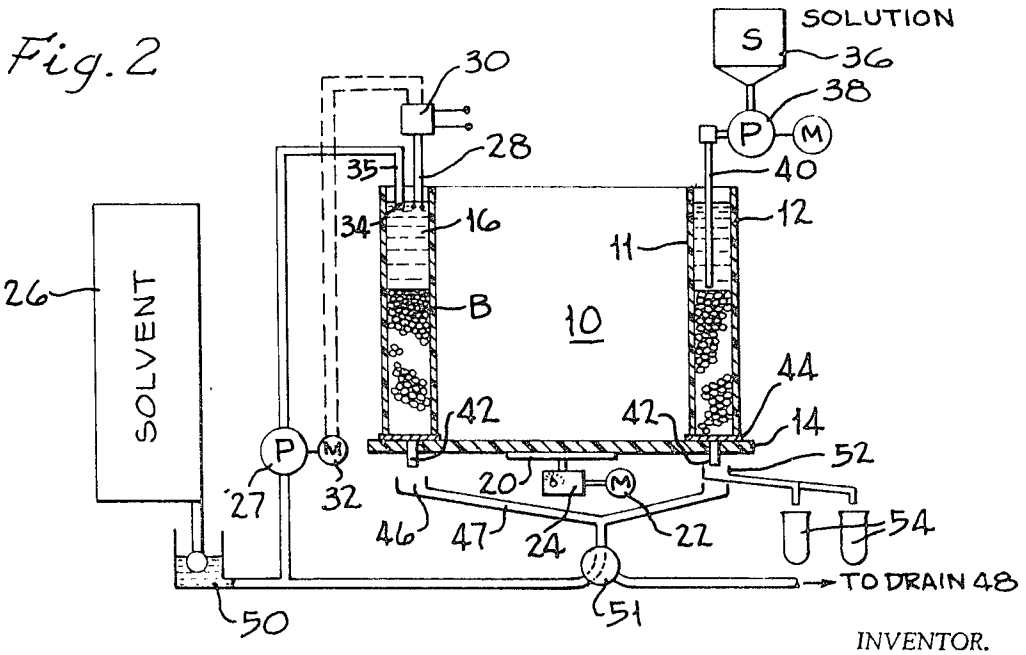


Fig. 2



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Fig. 3

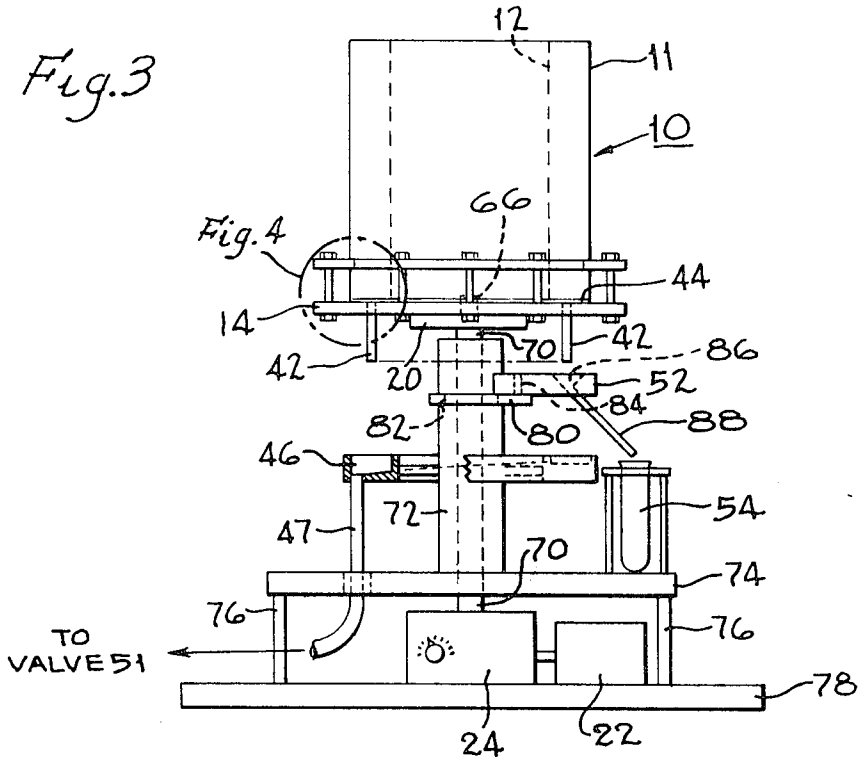


Fig. 4

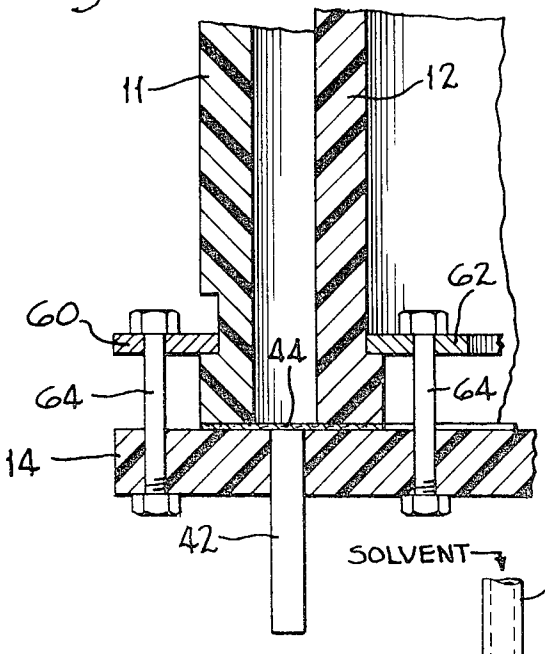


Fig. 5

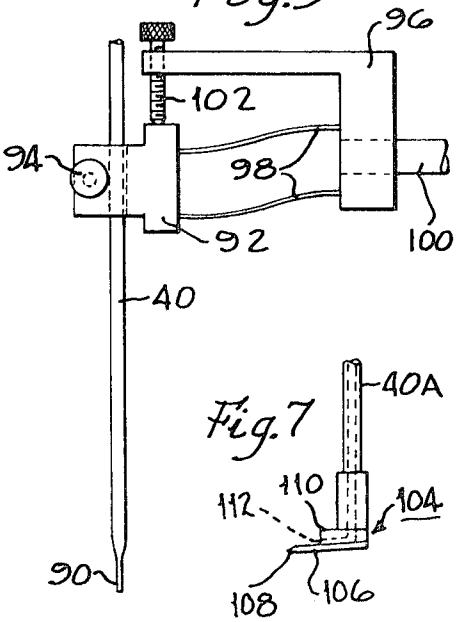
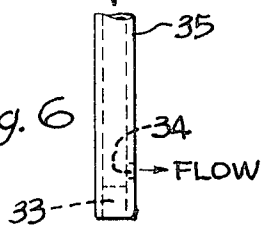


Fig. 7

Fig. 6



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APPARATUS FOR CONTINUOUS LIQUID-SOLID PHASE CHROMATOGRAPHY

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

This invention relates to an apparatus for continuous liquid-solid phase chromatography.

Although there has been much activity in chromatography and chromatographic techniques in recent years, very few, if any, new developments have been concerned with continuous liquid-solid phase chromatography. Many of the recent developments have been restricted to gas-solid phase devices and procedures.

An object of this invention is to provide an apparatus for liquid-solid phase chromatography that is relatively simple and inexpensive to construct, easy to use and continuous in operation.

A further object is to provide an apparatus in which the principle of continuous chromatography can be applied to the separation and purification of chemical compounds for the purpose of increasing yields and thus reducing costs of preparation.

In general, according to this invention the above objects are accomplished by an apparatus in which a column is formed by two concentric cylinders clamped to a bottom plate having exit orifices in the center of the column space between the two cylinders. The bottom plate and column assembly rest on a turn table operably connected to a drive means. The apparatus is equipped with a drain ring and a fraction collecting device. A unique accessory to the apparatus is a leveling plow which is a shoe-like attachment on the end of a hypodermic needle. The plow is used in the column preparation operation to obtain a flat fixed phase bed surface for even layering of the solution and uniform entry of solution into the fixed phase.

The apparatus will now be described in conjunction with the drawings wherein:

FIG. 1 pictorially illustrates the principle of this device.

FIG. 2 schematically illustrates the entire system including the pipeing and electrical control.

FIG. 3 is a side elevation of the actual construction.

FIG. 4 is a fragmentary sectional view taken in the region 4 of FIG. 3.

FIG. 5 is a side elevation of the solution needle and its adjustable support.

FIG. 6 is a side elevation of the solvent introducing tube.

FIG. 7 is a fragmentary side elevation of the bed leveling plow.

Reference to FIG. 1 will explain the principle of operation of this apparatus for continuous liquid-solid phase chromatography. The solution S is applied continuously to the top of a bed B of fixed phase which is contained in a chromatographic column 10 between two concentric hollow cylinders 11 and 12. The bed B is supported between the cylinders on a bottom plate 14 to which the cylinders are secured. A head of eluting solvent 16 is maintained above the fixed phase bed B. As the solution S and solvent 16 move down through the bed B, the component X (dashes) moves faster than component Y (circles). Consequently, rotation of the assembly causes a separation of the components X and Y and they can be separately withdrawn at openings in the bottom plate 14 to be hereinafter described. By introducing the solution continuously, the components are drawn off continuously.

The entire continuous system is best seen in FIG. 2. The chromatographic column assembly 10 is centered on a turntable 20 which is rotated by a motor 22 through a variable speed transmission 24.

After the fixed phase bed B is prepared, the technique of which will be later described, a continuous flow of solvent is provided from a vessel 26 by means of pump 27. A constant head is maintained by means of a probe 28 in the column operating a sensitive relay 30 which controls current to the pump motor 32. When the liquid level in the column drops, an electrical circuit is broken between two platinum electrodes in the probe 28. The relay 30 will then close a circuit to pump motor 32, and the solvent 16 is pumped into the column through an orifice 34, in tube or needle 35 (see FIG. 7), which directs the flow horizontally so as not to disturb the surface of the fixed phase B.

The solution S is applied smoothly onto the surface of the bed B from a vessel 36 by means of a low and adjustable flow rate pump 38. The needle 40 that discharges the solution will be described in greater detail in discussion of FIG. 5.

The bottom plate 14 of the column 10 is provided with a plurality of tubed aperatures or orifices forming drain tips 42 under the bed B. An annulus of coarse grade filter paper 44 is clamped between the bottom edges of the hollow cylinders 11 and 12 and the planar surface of bottom plate 14 to support the bed material at these apertures.

The effluent from the bottom of the column 10 drips into a drain ring 46. The liquid in the ring may be directed either to the drain 48 or back to a recirculating reservoir 50 to be pumped back onto the column, depending on the position of valve 51.

Once the input conditions are established and even and continuous flow rates achieved for both solvent and solution, a fraction collecting device 52 is placed under the appropriate drain tip 42 to divert the fractions X and Y to suitable containers 54.

Reference to FIGS. 3, 4, 5 and 6 will show the actual construction of the apparatus. The hollow cylinders 11 and 12 are tightly clamped to the bottom plate 14 by means of clamp rings 60 and 62 respectively which are secured to the bottom plate 14 by means of a plurality of machine screws 64. The filter paper 44, an annular sheet, is shown in position. The assembled column 10 is centered on the turntable 20 by means of a stud or sleeve 66 on the top of said turntable and closely fitting a center hole in the bottom plate 14. The turntable is supported on the output shaft 70, of variable speed transmission 24.

A stationary hollow cylinder or support column 72 encloses most of shaft 70 and is supported on a bridge member 74 to which it is secured. This bridge plate overlies the transmission 24 and motor 22 and is supported on posts 76 from a base plate or member 78. The drain ring 46 is supported on the cylinder 72 underlying the drip tips 42. A tube 47 conducts the solvent 16 collected in the ring to the valve 51 where it is conducted to the drain 48 or to the reservoir 50.

Above the drain ring 46 a rotatable platform or fraction collector support 80 is supported in a groove 82 in the cylindrical column 72. Pins 84 or other suitable means permit mounting the fraction collector 52 thereon. This collector has conical cavities 86 formed on its upper surface at intervals equal to the spacing of the drip tips 42 depending from bottom plate 14. In plan view, not shown, the fraction collector is a quadrant underlying one quarter of the drip tips 42 so that it can be located under those tips from which both fractions X and Y may flow. From each depression 86 a tube 88 is provided to conduct the fractions to suitable containers 54. The tubes from which only solvent 16 flows can be blocked off or diverted to the drain 48.

FIG. 5 illustrates the tube or needle 40 through which the solution is pumped onto the surface of the bed B. The tip 90 is flattened to provide a small flat orifice to prevent solution-solvent intermixture. This spreads the solution across the width of the bed B when the long dimension of the orifice is aligned with a radius of the column 10.

The tube 40 is supported in a clamp block 92 with a hand screw 94. Block 92 is connected to bracket 96 by means of two parallel flat springs 98. The bracket 96 is supported from any fixed base by arm 100. A hand screw 102 permits fine vertical adjustment without disturbing the perpendicularity of the tube 40.

FIG. 6 illustrates the lower end of the tube 35 through which the solvent is introduced. A plug 33 seals the end of the tube and an orifice 34 provides the horizontal flow previously discussed.

FIG. 7 illustrates the plow tip 104 of a tube 40A used to prepare the fixed phase bed B prior to operation. It is supported and adjusted in the clamp block 92 of FIG. 5. It is necessary to have an absolutely flat fixed phase bed surface for even layering of the solution and uniform entry of solution into a fixed phase. Consequently, a special device, the leveling plow, was developed. The plow tip or plow 104 consists of a shoe-like attachment on the end of tube 40A. A flat plate 106, sharpened on the leading edge 108, is soldered to the bottom of the shoe, the leading edge extending past the "toe" 110 of the shoe. A channel 112 extending almost the width of the shoe and five one-thousandths inch deep, connects to the bore of the needle and opens out toward the sharpened edge of the plow blade 106. The plow blade itself is titled downwards 10° out of a plane perpendicular to the needle. The shoe and blade are of suitable width so that the assembly will fit inside the column. To prepare the bed B for operation the column is rotated at 1.33 r.p.m. and the gel allowed to drain into the column from any suitable container. Then the solvent pump 27 is turned on to maintain the solvent head.

After the gel has settled, it is then necessary to level the top surface of the bed, which is done with the leveling plow 104 previously described. The plow is mounted in the block 92, and a micro-valve not shown connects the tube 40A to a solvent supply in the loading reservoir. The solvent flow is adjusted with the needle well above the gel bed. The plow is then lowered to just above bed B and clamped securely in position. The column is rotated with the rotator at 1.33 r.p.m., the solvent flow turned on gently, and the plow lowered into the bed surface. The blade of the plow lifts the top surface of the fixed phase bed and the jet of solvent disperses it. To remedy highly uneven packing of the bed it is sometimes desirable to turn on the solvent with some force and disperse the fixed phase with the jet. After the top surface has been dispersed, the flow of solvent is shut off and the column allowed to rotate. As the fixed phase resettles, the plow is lifted by degrees until the fixed phase no longer balls up in front of the plow. At this point the bed should be quite level, having neither high nor low points, and it should not slope either to the inside nor outside of the column. The plow is then withdrawn. This phase of the operation is most critical. Care should be exercised not to allow the plow to drop into the surface of the gel, for if it does a compressed area results and a distortion in the bands develops which will not disappear.

I claim:

1. Apparatus for continuous liquid-solid phase chromatography comprising:

a. a flat-circular plate having a plurality of orifices in its surface disposed in a circular array near the periphery of said plate;

b. a first open-ended cylinder tightly clamped at one end thereof to a surface of the plate;

c. a second open-ended cylinder of smaller diameter than said first cylinder similarly clamped to the same surface of the plate, said two cylinders being coaxial with each other and with the axis of the plate and being of such dimensions that the inner surface of the first cylinder and the outer surface of the second cylinder define between them an annular, open-top column over the circular array of orifices in the surface of the circular plate;

d. a liquid-permeable support means covering the circular array of orifices, said support means extending and tightly clamped between the surface of the circular plate and the ends of the two coaxial cylinders, said support means serving to support a bed of particulate adsorbent contained in the annular column defined by the two cylinders;

e. variable speed rotating means and an elongated shaft connecting said rotating means to the circular plate;

f. a stationary elongated sleeve surrounding said shaft;

g. a circular trough secured to said stationary sleeve disposed beneath the circular array of orifices in the circular plate to collect liquid draining through said orifices;

h. sample collecting means pivotally mounted on said stationary sleeve between the circular trough and the circular plate, said sample collecting means being pivotable to be positioned under the circular array of orifices to collect liquid draining therethrough;

i. means for transferring said collected liquid to a container;

j. separate means for supplying a solution and an eluting solvent to the top of the annular column;

k. means connected to the solvent supply means for maintaining a constant head of solvent; and

l. levelling means, secured to the solution supply means, having a leading edge opposed to the direction of rotation of the column and adapted to extend beneath the surface of the particulate adsorbent in said column whereby to level said adsorbent and prevent compacting of the surface.

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