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Ito et al.

[11] **3,856,669**

[45] **Dec. 24, 1974**

[54] **ELUTION CENTRIFUGE-APPARATUS AND METHOD**

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[73] Assignee: **The United States of America as represented by the Secretary of the Department of Health, Education and Welfare,** Washington, D.C.

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[21] Appl. No.: **375,882**

[52] **U.S. Cl.** .... **210/31 C,** 210/198 C
[51] **Int. Cl.** **B01d 15/08**
[58] **Field of Search** ...... 210/31 C, 198; 55/67, 197, 55/386

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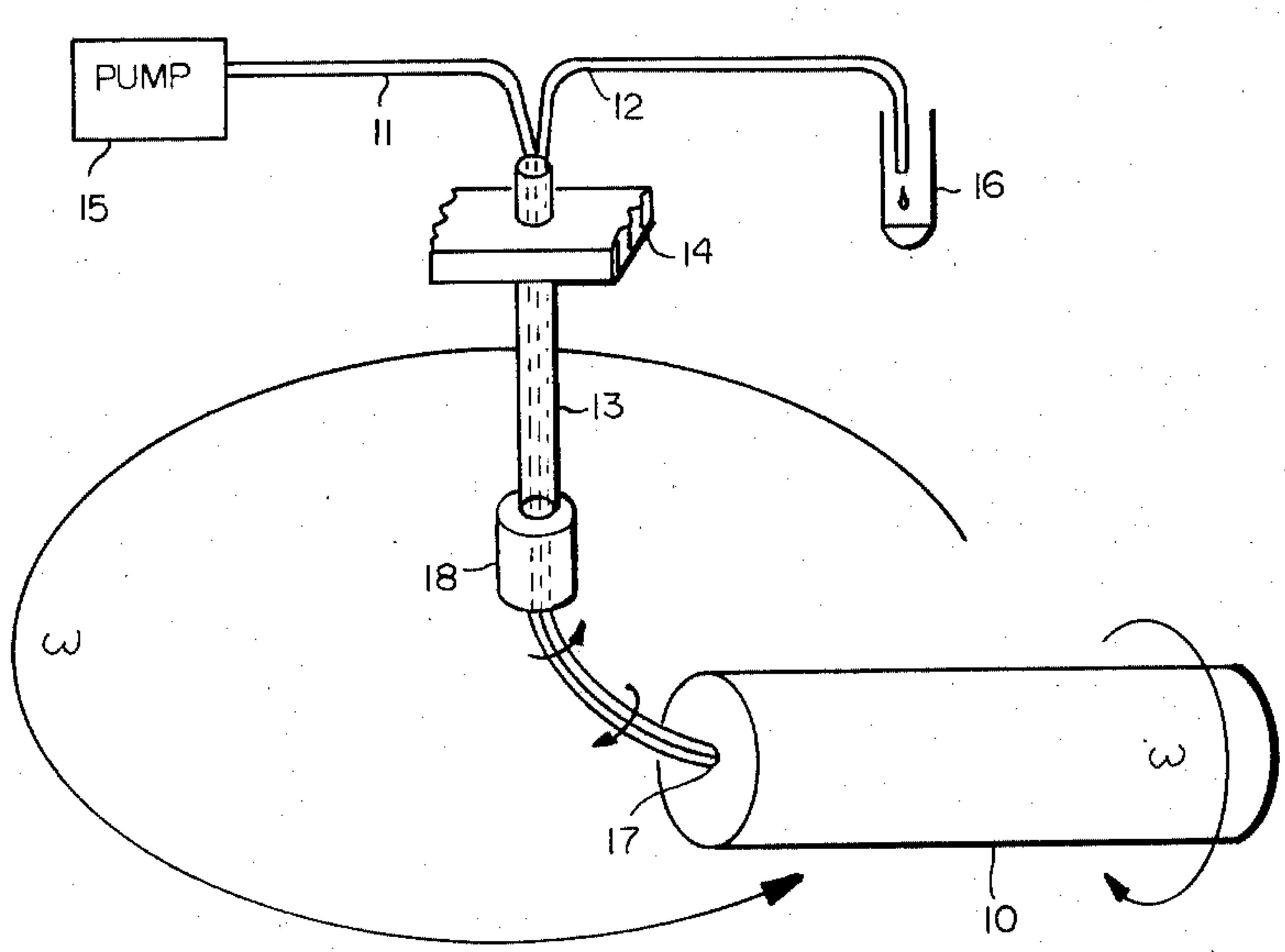
Primary Examiner—John Adee

[57] **ABSTRACT**

An apparatus for elution centrifugation used in countercurrent chromatography comprises a high speed centrifuge head which revolves around a central vertical axis, a cylindrical column holder which is horizontally and rotatably carried by the centrifuge head, and a separation column fixedly disposed within the holder. The separation column includes a fine tube which passes externally introduced fluids to and from the centrifuge head and the cylindrical column holder without the use of rotating seals. The seals are eliminated by rotating the cylindrical column holder about its own axis while, simultaneously, revolving it around the central vertical axis of the apparatus at the same angular velocity.

9 Claims, 12 Drawing Figures



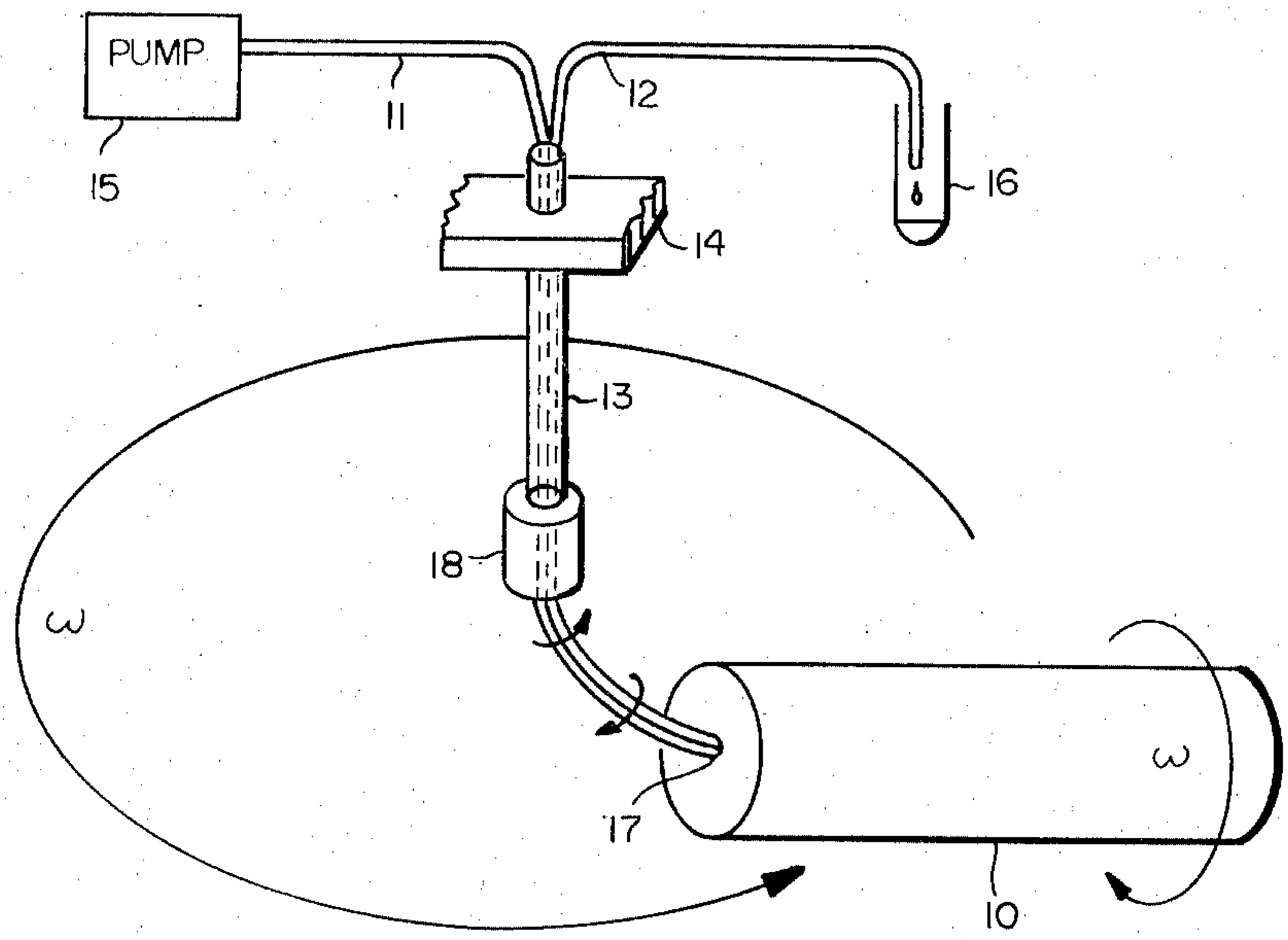


FIG. 1

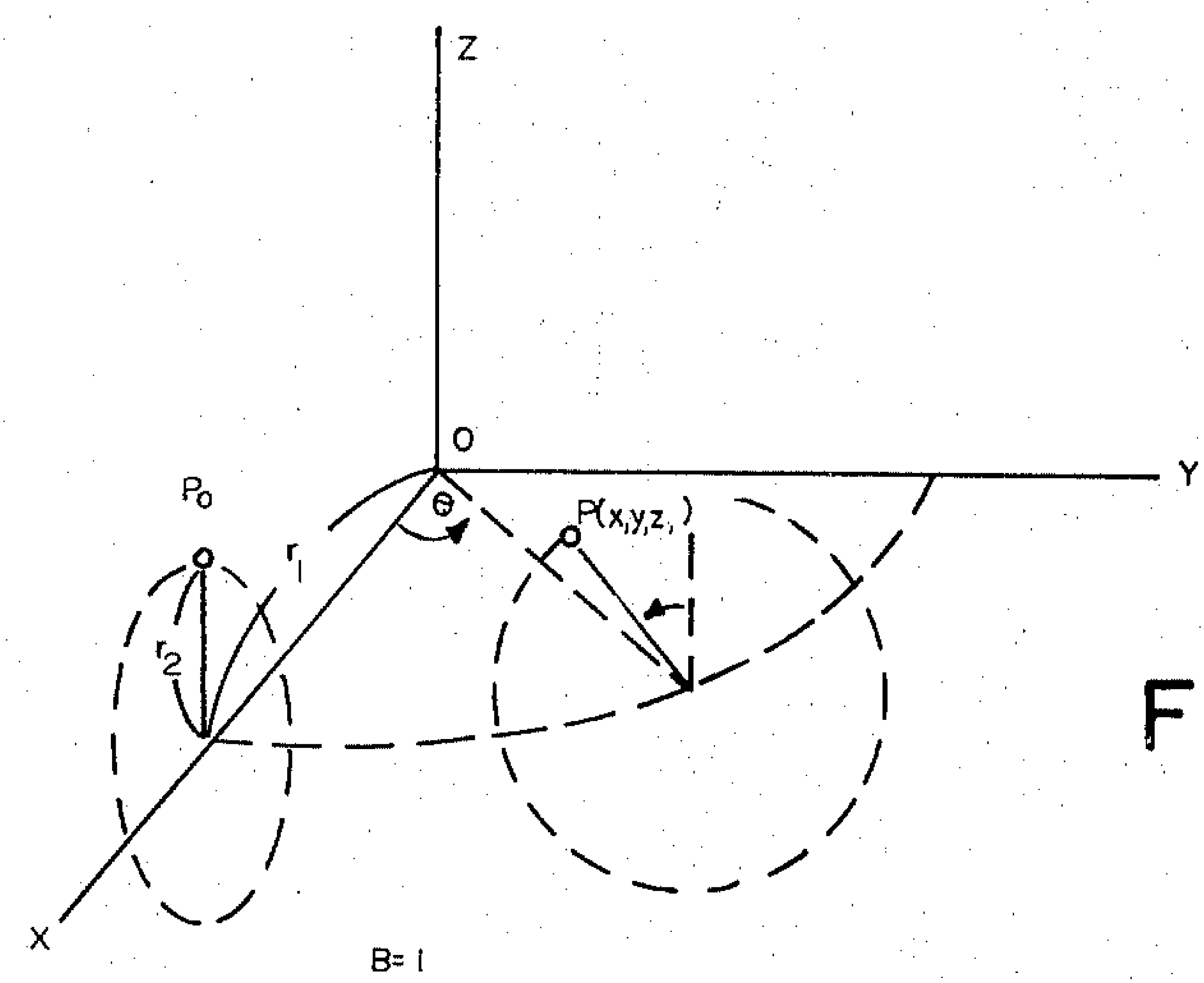


FIG. 2

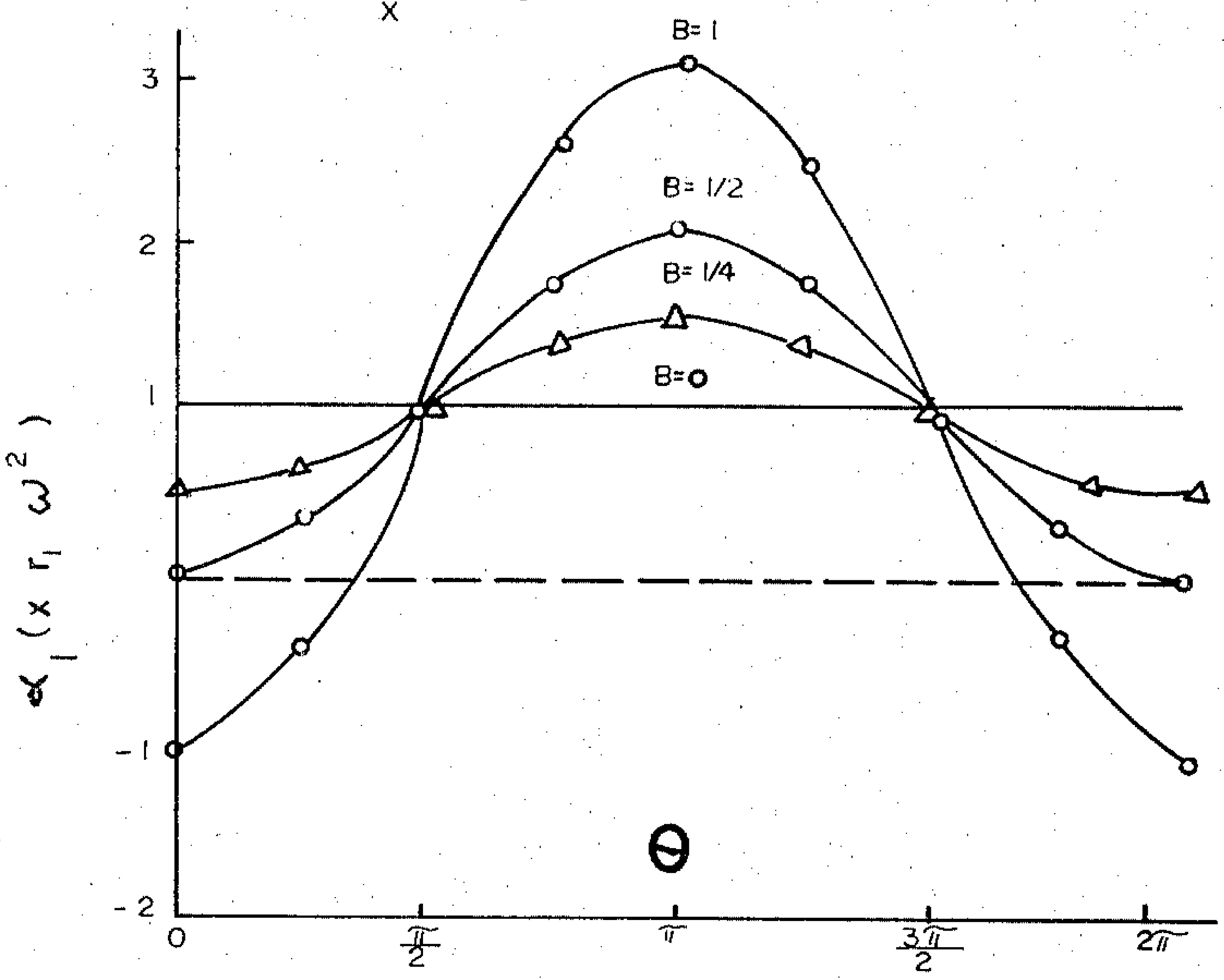


FIG. 3

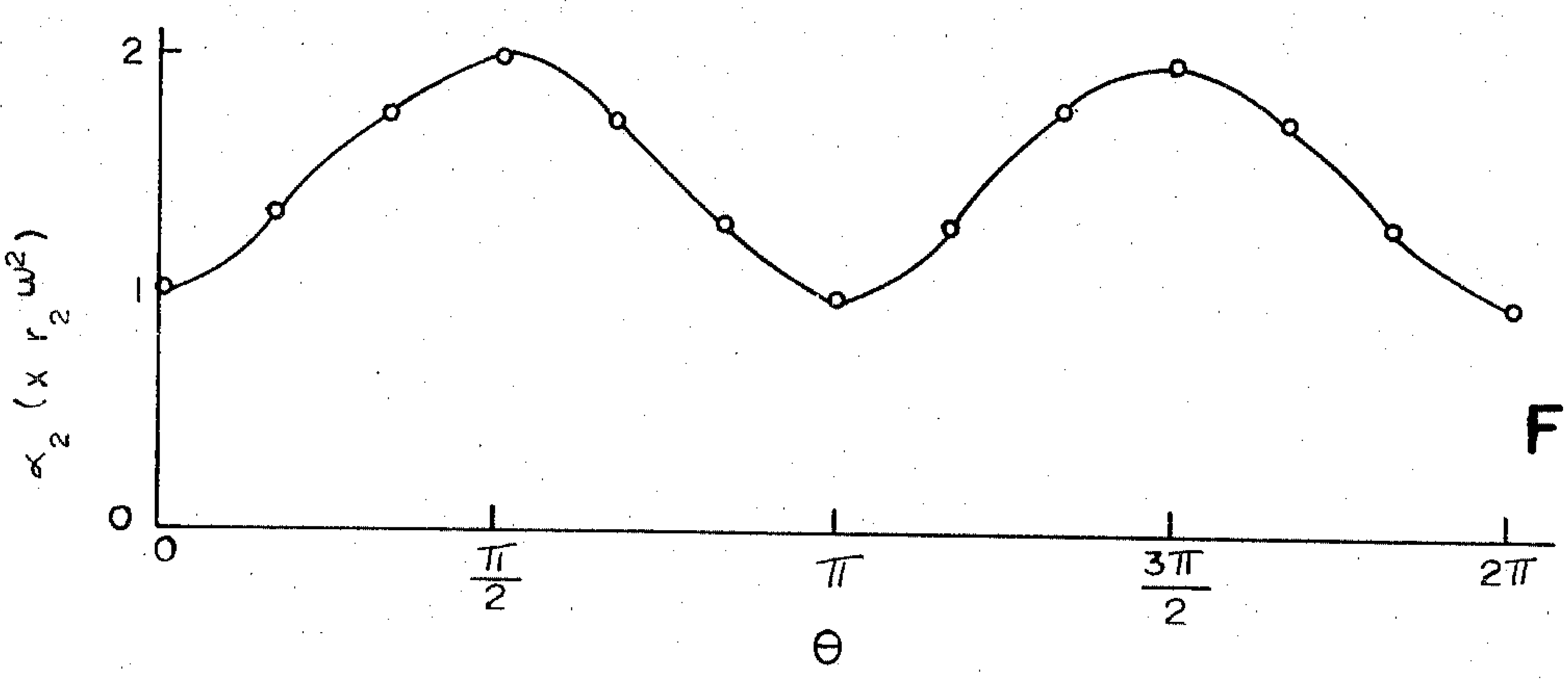


FIG. 4

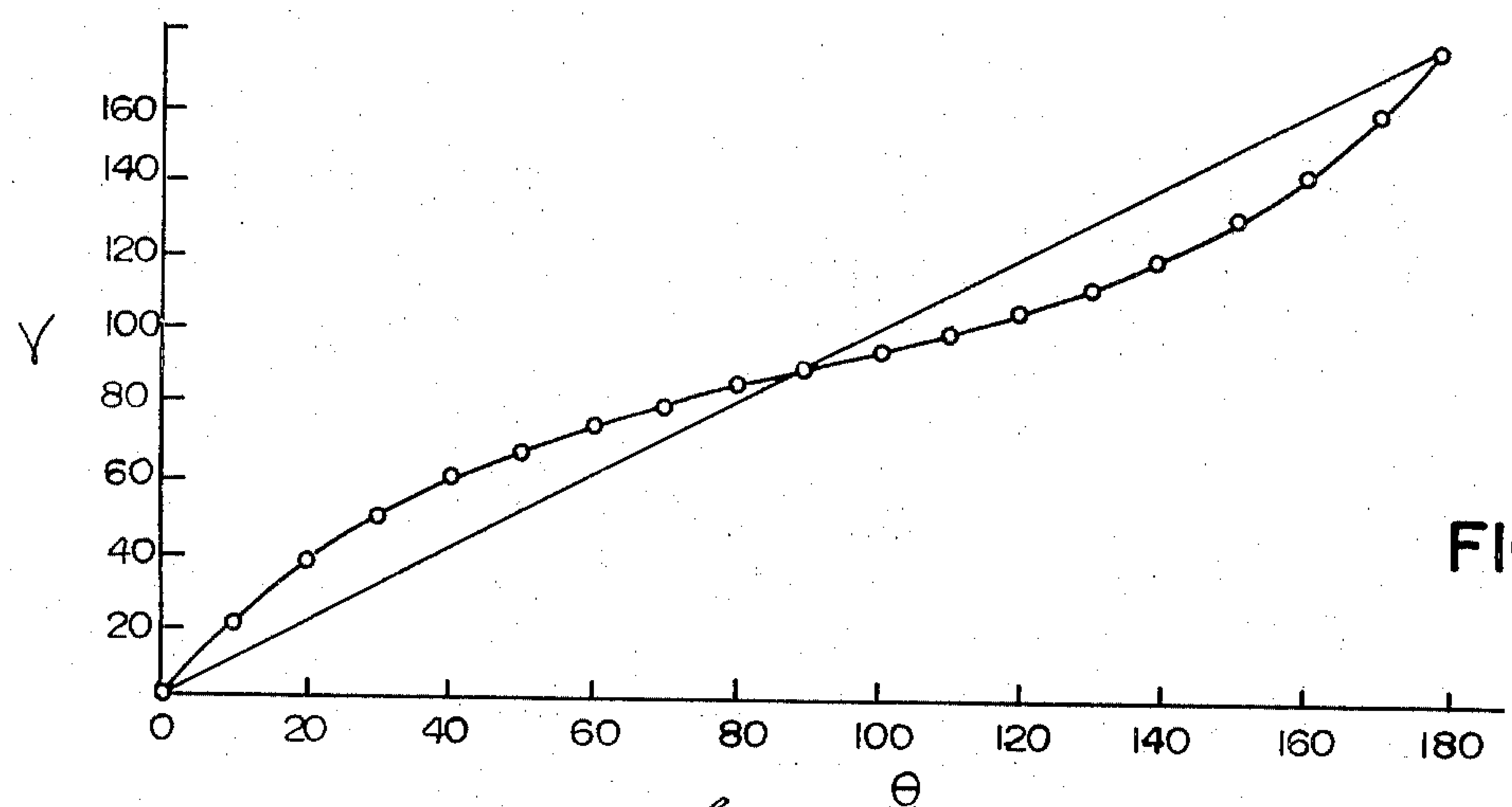


FIG. 5

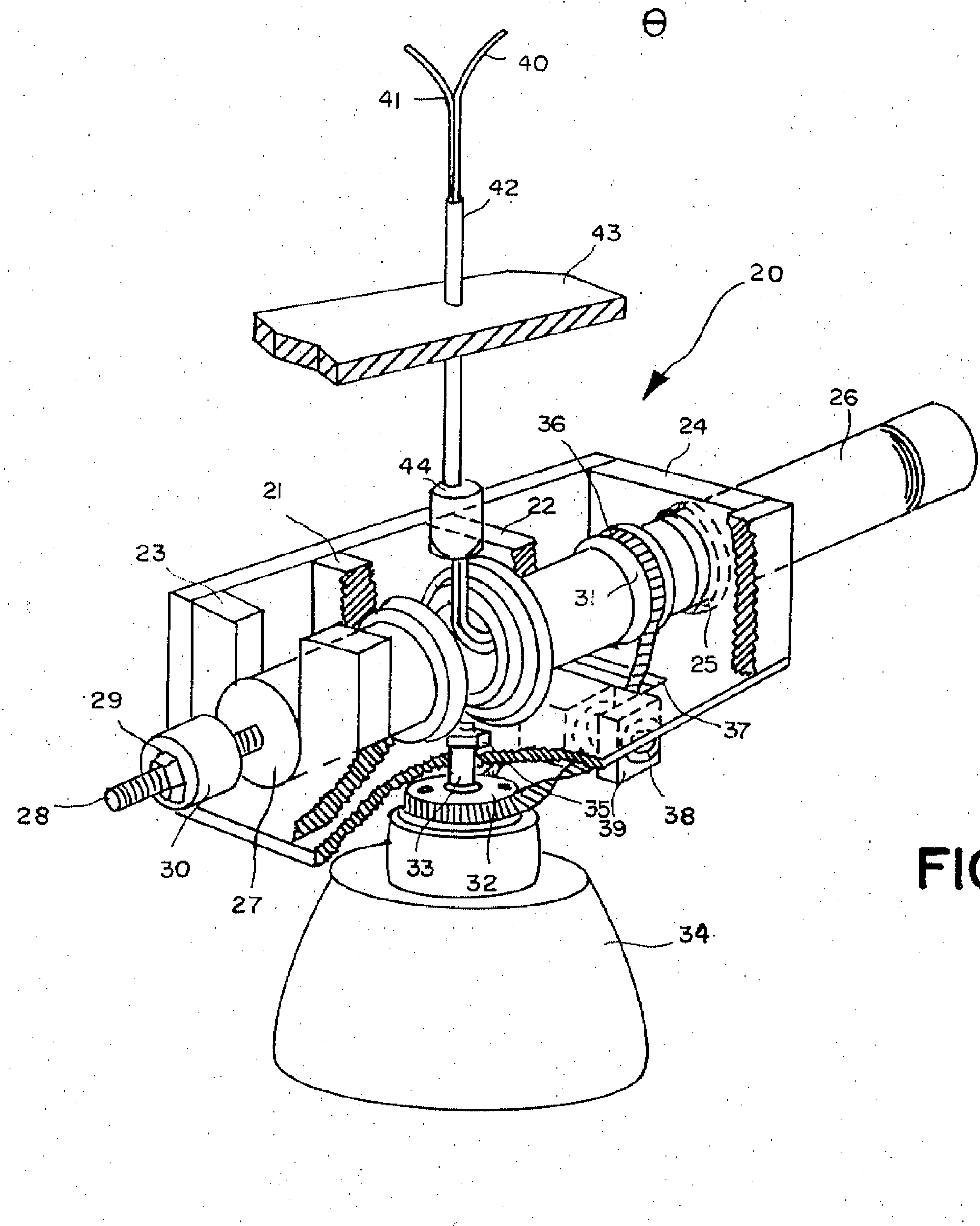


FIG. 6

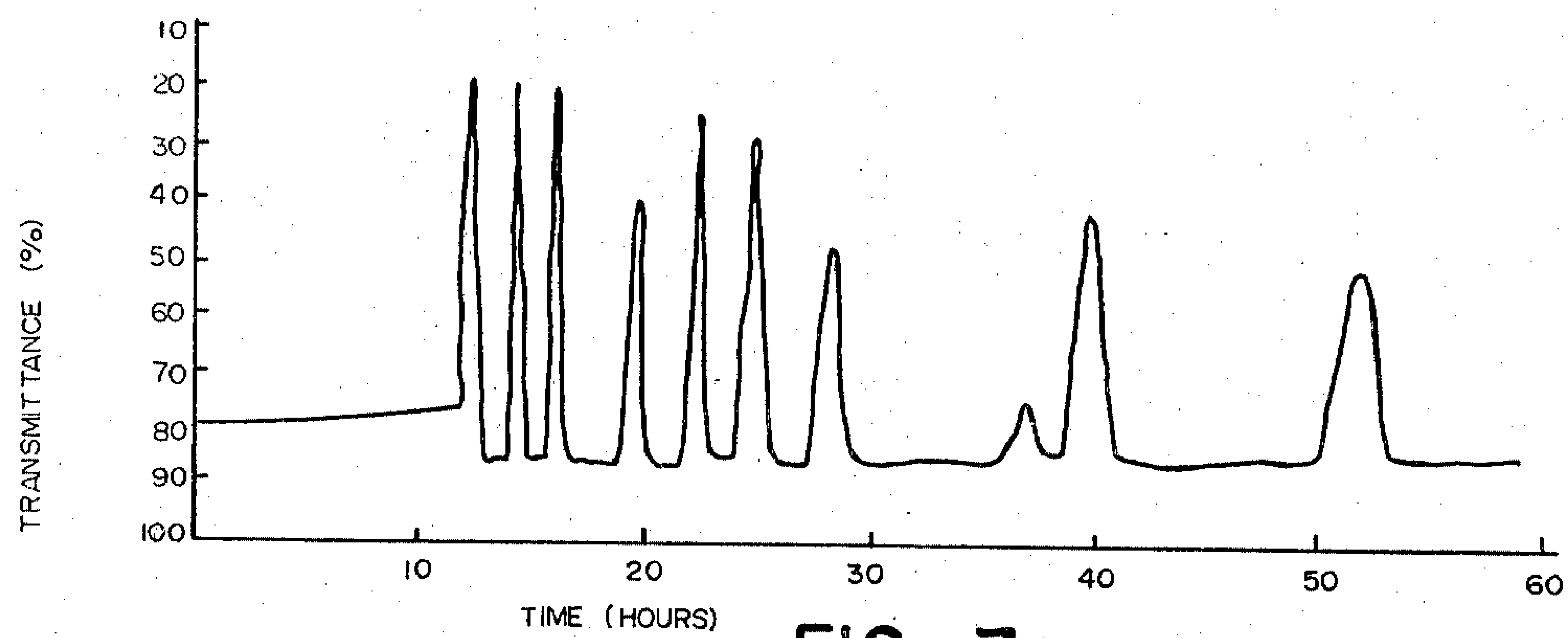


FIG. 7

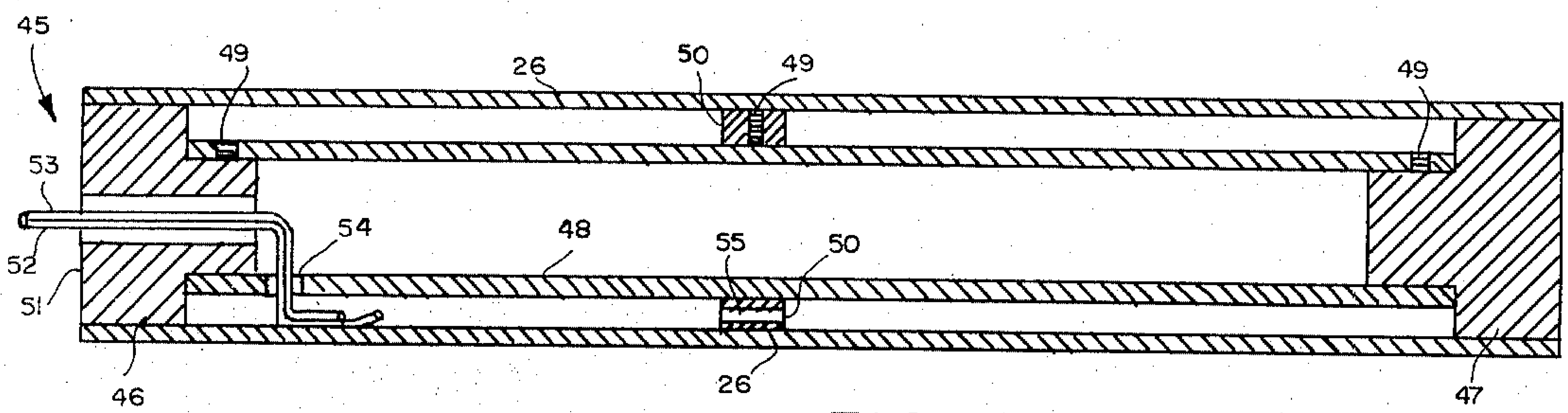


FIG. 8

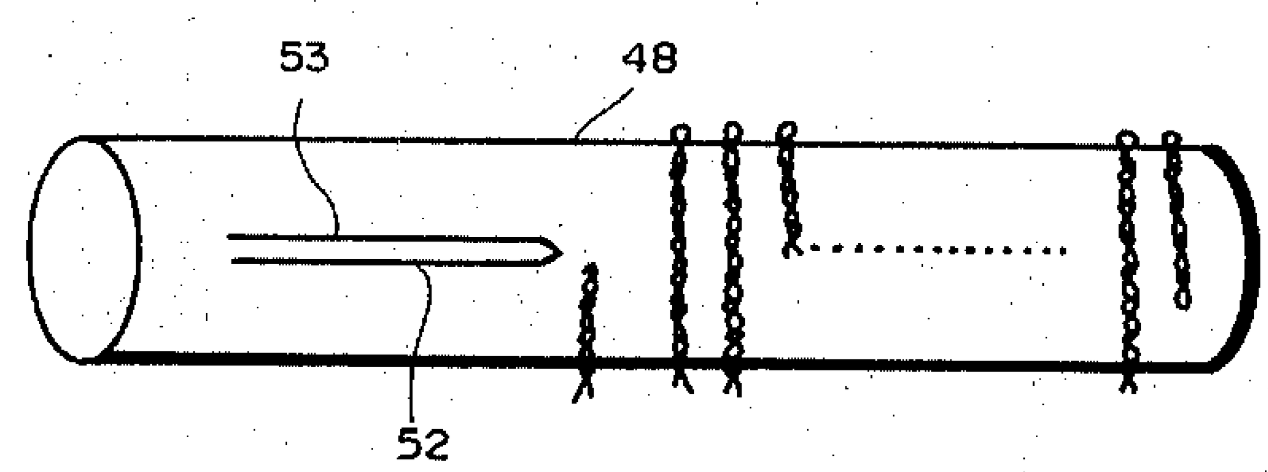


FIG. 9

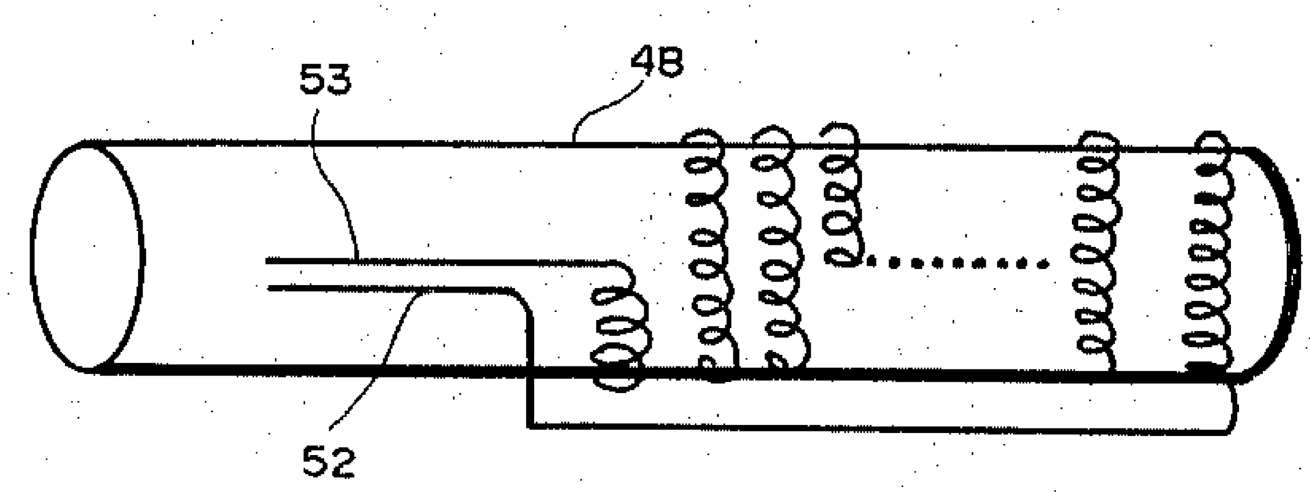


FIG. 10

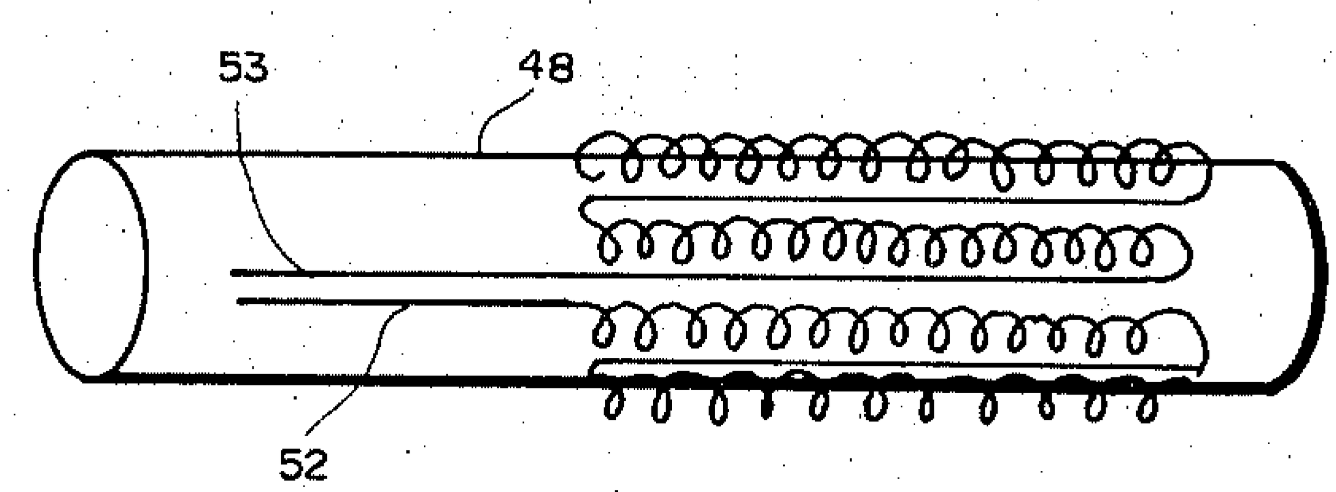


FIG. 11

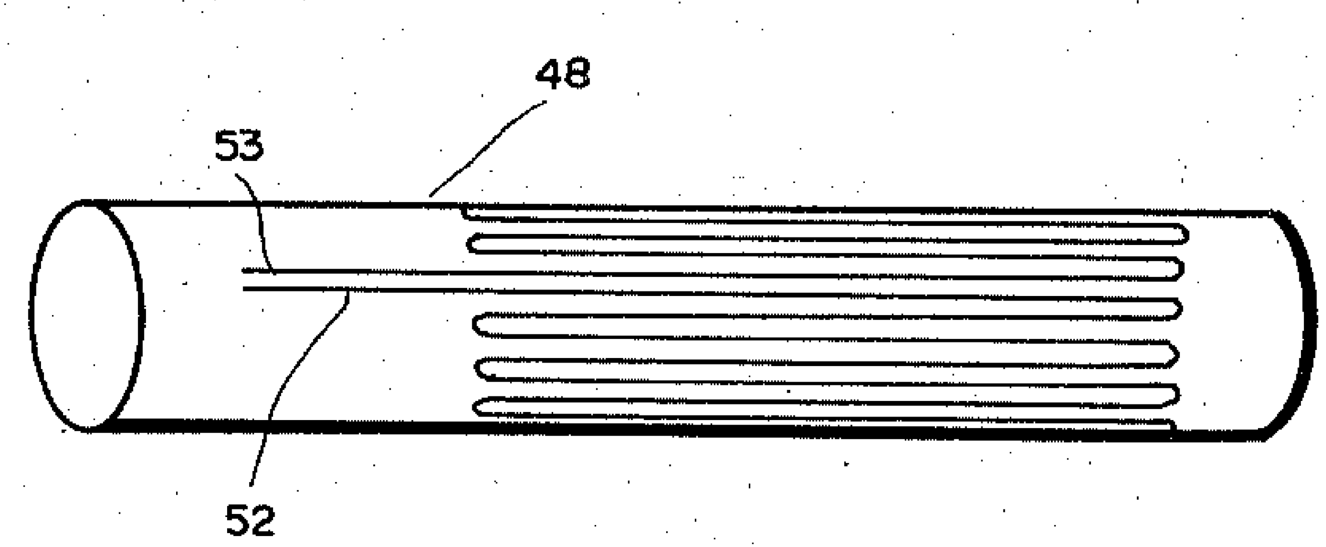


FIG. 12

ELUTION CENTRIFUGE-APPARATUS AND METHOD

FIELD OF THE INVENTION

The present invention relates to the separation of samples and, more pertinently, to an elution centrifuge method and device which permits elution and elutriation on both analytical and preparative scales.

BACKGROUND OF THE INVENTION

Various separation techniques are known in which two immiscible or partially soluble liquid phases are brought into contact for the transfer of one or more components. Among such liquid-liquid or solvent extraction techniques are partition chromatography and countercurrent chromatography. This latter technique can be carried in various ways (see our copending application Ser. No. 275,777 now U.S. Pat. No. 3,775,309) among which is helix countercurrent chromatography, in which a horizontal helical tube is filled with one phase of a two-phase liquid and the other phase is introduced at one end of the helix and passes through the first phase. To enable the countercurrent process to take place inside a very small diameter tube having a maximum number of turns, it is desirable to enhance the gravitational field by the use of a centrifuge.

Prior art helix countercurrent chromatography devices using a centrifuge employ rotating syringes or rotating seals. A major problem with these devices is that the rotating syringes or seals make gradient or stepwise elution difficult, if at all possible, and, therefore, decrease the efficiency of the devices.

The efficiency of prior art flow-through coil planet centrifuges also suffers because they provide for a non-universal application for solvent systems such as polymer phase systems used for the separation of macromolecules and particulates.

SUMMARY OF THE INVENTION

It is, accordingly, an object of the present invention to overcome the defects of the prior art, such as indicated above.

Another object is to provide for improved separation of one material from another.

Another object of the present invention is to provide a centrifuge capable of elution and elutriation on both analytical and preparative scales.

Another object is to provide an elution centrifuge capable of various applications including countercurrent chromatography.

Another object is to provide an elution centrifuge that yields a resolving power at least equivalent to that in prior art helix countercurrent chromatography devices.

A further object is to provide an elution centrifuge for countercurrent chromatography which has a higher efficiency than that of refined gas chromatography.

In furtherance of these and other objects, a principal feature of the present invention is an elution centrifuge which eliminates rotating seals, thereby permitting an accurate flow through the feed tube under high feed pressure and preserving the narrow bands of the separated samples in passing through the negligibly small dead space of the return tube. Another feature is the particular fluctuation of the centrifugal acceleration field in which stability of the field is a function of radii of both revolution and rotation. Thus, the shortcomings of prior art centrifuges used for countercurrent chromatography are satisfactorily overcome by the present invention.

The elution centrifuge of the present invention is characterized by a flow-through separation column including fine lead tubes without rotating seals. The device also includes a cylindrical column holder, the holder being horizontally and rotatably disposed in a centrifuge head which revolves around a central vertical axis. The column holder revolves around the vertical axis while, simultaneously, rotating around its own horizontal axis at the same angular velocity and in a direction which avoids any twisting of the lead tubes caused by revolution.

BRIEF DESCRIPTION OF THE DRAWING

FIG. **1** is a schematic diagram of the elution centrifuge.

FIG. **2** is a graph showing the motion of an arbitrary point on the centrifuge column holder in the *x-y-z* planes.

FIG. **3** is a graph showing the fluctuation of the centrifugal acceleration of the arbitrary point parallel to the axis of the holder of FIG. **2** during one revolution.

FIG. **4** is a graph showing the fluctuation of the centrifugal acceleration of the arbitrary point in a plane perpendicular to the axis of the holder of FIG. **2** during one revolution.

FIG. **5** is a graph showing the undulation of the direction of the centrifugal acceleration of FIG. **4** during half a revolution.

FIG. **6** is a perspective view of a preferred embodiment of the elution centrifuge.

FIG. **7** is a cross-sectional view of the separation column and column holder shown in FIG. **6.**

FIG. **8** is a perspective view of a separation column and a stretched helix tube arrangement.

FIG. **9** is a perspective view of a separation column and a coiled helix tube arrangement. column and a parallel helix tube arrangement.

FIG. **11** is a perspective view of a separation column and a loop-type tube arrangement.

FIG. **12** is a graph of the results of dinitrophenyl (DNP) amino acid separation achieved through the use of the device of FIG. **6.**

For a better understanding of the invention a possible embodiment thereof will now be described with reference to the attached drawing, it being understood that this is to be intended as merely exemplary and in no way limitative.

DETAILED DESCRIPTION

The principle of the system is illustrated in FIG. **1.** A cylindrical holder **10** containing a separation column (not visible) is held horizontal. Both feed and return tubes **11** and **12,** respectively, pass through an axial hole **17** of the holder **10** and are wrapped around the separation column. The tubes **11** and **12** are supported tightly at the center of the apparatus by a guide tube **13** fixed to the top frame **14** of the centrifuge. The holder **10** revolves around the central vertical axis of the apparatus in the horizontal plane and simultaneously rotates about its own horizontal axis at the same angular velocity. The relative directions of the revolution and rotation are shown by the arrows in FIG. 1, it being understood that both revolution and rotation may be in the direction opposite to that shown so that twisting of the tubes 11 and 12 is avoided. This simultaneous rotation and revolution at the same angular velocity is achieved by coupling a pulley fixed to the holder by means of a toothed belt to a stationary pulley of equal diameter on the axis of the centrifuge drive (e.g. see description below of FIG. 6). This synchronous rotation unwinds the twist of the lead tubes caused by the revolution of the holder 10.

A pump 15 supplies fluid through the feed tube 11 to the holder 10. After centrifuging the fluid travels through return tube 12 to a receptor 16. A polytetrafluoroethylene collar 18 located at the lower end of the guide sleeve 13 protects the feed tube 11 and return tube 12 throughout their freely moving portion, thereby, extending their life expectancy. A piece of silicon rubber tubing (not shown) is inserted in the tapered hole of the polytetrafluoroethylene collar 18 to prevent the feed and return tubes from directly contacting the polytetrafluoroethylene collar.

The synchronous rotation also adds a peculiar effect to the centrifugal force field at the column holder. A simple mathematical analysis, introduced below, shows that any arbitrary points in the column holder, except for those located on the axis of rotation, are subjected to a periodic fluctuation of the centrifugal force.

Referring now to FIG. 2, an arbitrary point, P (x,y,z), is located at r_1 from the axis of revolution and at r_2 from the axis of rotation at the starting point P_o. The point then revolves around the z axis at angular velocity ω and simultaneously rotates around the column axis in the x-y plane at the same angular velocity. Consequently, point P travels always on a spherical surface centered at point O with a radius of $(r_1^2 + r_2^2)^{1/2}$, and at time t, (since $\theta = \text{wt.}$)

$$x = r_1 \cos\theta + r_2 \sin^2\theta$$

$$y = r_1 \sin\theta - r_2 \sin\theta \cos\theta$$

$$z = r_2 \cos\theta$$

Since the net centrifugal acceleration field,

$$\alpha = [(d^2x/dt^2) + (d^2y/dt^2) + (d^2z/dt^2]^{1/2}$$

gives a rather complicated picture of the three-dimensional change of its direction with θ, it is convenient to express it in terms of two components, α_1 (centrifugal acceleration parallel to the axis of the holder), and α_2 (centrifugal acceleration in a plane perpendicular to the axis of the holder).

The first component is given by the equation,

$$\alpha_1 = -(d^2x/dt^2) \cos\theta - (d^2y/dt^2) \sin\theta$$

which reduces to

$$\alpha_1 = r_1\omega^2 (1 - 2\beta \cos\theta)$$

where $\beta = r_2/r_1$ and $r_1 \neq 0$.

Referring now to FIG. 3, the values of α_1 expressed in terms of $r_1\omega^2$ is plotted against angle θ during one revolution. Four lines are drawn according to the β values of 1, ½, ¼ and 0 as indicated. It illustrates that α_1 oscillates around the $\beta = 0$ line once in each revolution with amplitude dependent upon the β value. When β exceeds ½, α_1 crosses below the dotted 0 line, indicating that the acceleration acts momentarily in the opposite direction to cause the liquids to move toward the center of revolution. As β decreases, the amplitude of the oscillation becomes smaller and finally reduces to zero at $\beta = 0$ providing a stable acceleration field similar to that in the conventional centrifuge system.

The second component which acts in the plane perpendicular to the axis of the holder is given by the equation,

$$\alpha^2_2 = [(d^2x/dt^2) \sin\theta - (d^2y/dt^2) \cos\theta]^2 + (d^2z/dt^2)^2$$

or

$$\alpha_2 = r_2\omega^2(1 + 3 \sin^2\theta)^{1/2} = r_2\omega^2 [(5 - 3 \cos 2\theta)/2]^{1/2}$$

Referring now to FIGS. 4 and 5, FIG. 4 shows the relationship between α_2 (in terms of $r_2\omega^2$) and θ during one rotation. Note that α_2 oscillates twice in each revolution between the values of $r_2\omega^2$ and $2r_2\omega^2$, and that the wave form is not accurately sinusoidal. The value of α_2 is independent of r_1 and reduces to 0 at $r_2 = 0$. The acting direction (γ) of α_2 in the plane perpendicular to the column axis is calculated by the equation.

$$\tan\gamma = [(d^2x/dt^2) \sin\theta - (d^2y/dt^2) \cos\theta]/(-d^2z/dt^2)$$

which becomes

$\tan\gamma = 2 \tan\theta$ indicating that the direction of α_2 undulates about the 0 line twice in one rotation (see FIG. 5).

The results of the above analysis show that the synchronous rotation causes an oscillating centrifugal acceleration. The relative amplitude of the oscillation becomes greater as the distance (r_1) from the axis of revolution decreases or the distance (r_2) from the axis of the rotation increases. Thus, a stable centrifugal acceleration field can be obtained at or near the axis of the holder with a great radius (r_1) of revolution. On the other hand, the mixing or vibration, if desired, can be attained at the location remote from the axis of the holder with a small radius (r_1) of revolution.

FIG. 6 shows the preferred embodiment of a device in accordance with the instant invention. A centrifuge head 20, which is a substantially rectangular box defined by four wall plates including short side plates 23 and 24, may be made of aluminum or any other suitable material. Vertical septums 21 and 22 are located inside the head 20 between and parallel to the short side plates 23 and 24. The spacing between side plate 23 and septum 21, between septum 21 and septum 22, and between septum 22 and side plate 24 is substantially equal. The centrifuge head 20 which is shown in FIG. 6 with an open top may also be closed if desired and may be constructed by modifying a conventional centrifuge, such as the Model II manufactured by the International Equipment Company.

On the column side (right side in FIG. 6) a thrust bearing (not shown) and an ordinary ball bearing 25 are located within the septum 22 and short side plate 24, respectively, to support a cylindrical separation column holder 26 which extends horizontally through the ball bearing encircled opening in the side plate 24.

The septum 21 and short side plate 23 include U-shaped openings for horizontally supporting a counterweight 27 made of an aluminum cylinder or other suitable material. The counterweight 27 includes a threaded rod 28 which extends, substantially perpendicular, from the flat outer surface thereof. An adjustable bolt 29 and an adjustable weight 30 are mounted on the threaded rod 28.

The synchronous rotation of the separation column holder 26 is accomplished by a system of toothed pulleys of the same diameter including the pulleys 31 and 32. One collar-like pulley 31 is fixedly attached about the outer surface of the holder 26, approximately midway between the septum 22 and the side plate 24. A shaft 33 for revolving the centrifuge passes from the electric driving motor (not shown), protected within a housing 34; and the other stationary pulley 32 is fixedly attached to the top surface of the motor housing 34 coaxially about the rotating central shaft 33, thereby allowing the shaft 33 to rotate freely from the stationary pulley 32. The shaft 33 passes through the approximate center of the substantially rectangular bottom plate of the centrifuge head 20 which is securely fastened to the shaft 33 by a nut 35 or any other suitable fastening means.

The pulleys 31 and 32 are coupled by a toothed endless belt 36 which passes through a hole 37 in the bottom plate of the centrifuge head 20 and over a pair of toothed idler pulleys 38 (only one being visible in FIG. 6) mounted on both ends of a block 39 which is fixedly attached to the bottom plate of the centrifuge head 20. These idler pulleys 38 function to change the direction of the endless belt by 90 degrees.

Feed and return tubes 40 and 41, respectively, which may be made of polytetrafluoroethylene or other suitable material pass through a vertical guide sleeve 42 and are led through a center hole in the end of a separation column (not shown in FIG. 6) around which they are wound. The guide sleeve 42 passes through and is fixedly attached to the top of the centrifuge frame 43. A polytetrafluoroethylene collar 44 located at the lower end of the guide sleeve 42 protects the feed tube 40 and return tube 41 throughout their freely moving portion thereby preserving their life expectancy for many hours at the maximal speed of approximately 2,000 rpm. A piece of silicon rubber tubing (not shown) is inserted in the tapered hole of the polytetrafluoroethylene collar 44 to prevent the feed and return tubes from directly contacting the polytetrafluoroethylene collar.

FIG. 7 shows a cross section of the separation column holder 26 and a separation column 45 contained therein. The separation column 45 includes two circular T-shaped end plates or plugs 46 and 47. The smaller diameter ends of these plugs 46 and 47 are press fitted into the open ends of a cylindrical pipe 48. Located on the pipe 49 approximately midway between the end plates 46 and 47 is an O-shaped collar 50 which helps to support the center of the pipe 48. The collar 50 and end plates 46 and 47 are secured to the pipe 48 by set screws 49.

End plate 46 which faces towards the center of the centrifuge includes a central aperture 51 through which pass the feed and return tubes 52 and 53 from outside the separation column 45 through the aperture 51 in end plate 46 to the interior of the pipe 48. A hole 54 in the wall of pipe 48 allows the feed and return tubes to pass from the interior to the exterior thereof. The feed and return tubes are wound about the exterior surface of pipe 48 in one of many configurations which will be described hereinafter. Collar 50 includes a longitudinal hole 55 through which the feed and return tubes pass so that they may be wound about the entire length of the pipe 48.

The larger diameter of the circular T-shaped end plates 46 and 47 and the outer diameter of collar 50 are approximately equal to the inner diameter of the column holder 26 thereby allowing the separation column assembly 45 to be securely press fitted into the column holder 26. The feed and return tubes 52 and 53 are accordingly protected within the annular space between the pipe 48 and the column holder 26.

Referring now to FIGS. 8 - 11, it is seen that the feed and return tube arrangement may take various forms. FIG. 8 shows the tubing which is folded in two to form the feed and return tubes 52 and 53, respectively. The tubes are then twisted together to make between 10,000 and 20,000 turns and stretched in a helical pattern about the cylindrical pipe 48. The feed and return tubes are anchored at the end of pipe 48 fartherst from the central aperture 51 by a pin 54 which extends radially outwardly from the surface of the pipe 48.

FIG. 9 shows the feed and return tubes 52 and 53 wrapped about the pipe 48 in a coiled helix pattern. FIG. 10 shows the coiled feed and return tubes 52 and 53 wrapped about the pipe 48 in a parallel helix configuration. FIG. 11 shows the feed and return tubes 52 and 53 in a parallel loop configuration.

FIG. 12 shows the result of dinitrophenyl (DNP) amino acid separation acheived with the instant device and described more fully below.

In order to more fully describe the operation of the apparatus and the method as applied to countercurrent chromatography, separation of nine dinitrophenyl (DNP) amino acids on a two phase system composed of chloroform, glacial acetic acid and 0.1 N HCl (2:2:1) will now be discussed. The separation column is filled with the stationary lower (heavier) phase of the solvent and 3 μl of sample solution (solute), containing each component at about 1% where solubility permits, is introduced through the feed tube. The immiscible upper (lighter) phase of the solvent is pumped with any suitable syringe drive, such as Model 933 manufactured by the Harvard Apparatus Co. at a rate of 120 μl per hour while the apparatus is spun at 700 rpm at room temperature. It should be noted that the order of introduction of the heavier and lighter solvents may be reversed if desired.

The continued injection of the upper moving phase causes it to percolate through the stationary lower phase which is trapped by gravity and centrifugal force. Sample solution or solute which is introduced into the device as described above is, thereby, exposed to each stationary segment, attaining a degree of equilibration dependent upon the degree of mixing that results from the percolation, filming, and surface tension changes as the solute is partitioned between the phases. Consequently, such a solute introduced into the device is subjected to a partition process between the oscillating alternate segments of the two phases and finally eluted out through the return tube of the separation column. The eluate may be monitored by an LKB Uvicord II or similar device at 280 mm.

As shown in FIG. 12, nine DNP amino acids are eluted out with 54 hours. The efficiency of the present method ranges between 10,000 and 6,400 theoretical plates estimated according to the formula used in gas chromatography and indicates that a resolving power at least equivalent to that reported in helix countercurrent chromatography may be achieved.

The foregoing description of the specific embodiment will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify such specific embodiment and/or adapt it for various applications without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiment. It is to be understood that the phraseology or terminology employed herein is for the purposes of description and not of limitation.

What is claimed is:

1. An elution centrifuge comprising:

a separation column the axis of which is generally perpendicular to a main axis of revolution;

a feed tube for introducing fluids to said separation column and means for passing fluid to said feed tube;

a return tube for discharging fluids from said separation column and means for receiving fluid therefrom;

centrifugation means for revolving said separation column about the main axis of revolution and for simultaneously rotating said separation column about its axis at the same angular velocity to prevent twisting of said feed and return tubes; and

a stationary guide means, located outside said centrifugation means coaxial with the main axis of revolution, for guiding said feed and return tubes.

2. The device of claim **1** wherein said centrifugation means includes:

a drive shaft located on said axis of rotation;

a motor for rotating said drive shaft; and

a centrifuge head perpendicularly attached to said drive shaft, said centrifuge head comprising at one end a separation column holder rotatable about the axis of rotation of said separation column and an adjustable counterweight located at the opposite end.

3. The device of claim **2** wherein said centrifugation means for simultaneously rotating said separation column further includes:

a first drive means coaxially located about said drive shaft;

a second drive means engaging said rotatable separation column holder and disposed in a plane perpendicular to said first drive means;

an endless belt connecting said first and second drive means; and

means disposed on both sides of said first and second drive means for changing the direction of said endless belt by 90°.

4. The device of claim **3** wherein said first and second drive means are pulleys of equal diameter.

5. The device of claim **4** wherein said means for changing the direction of the endless belt are idler pulleys, said idler pulleys being rotatable about an axis defined by the intersection of the planes of rotation of said pulleys and spaced apart a distance approximately equal to the diameter of the pulleys.

6. The device of claim **1** wherein said stationary guide means comprises:

a cylindrical hollow sleeve; said sleeve having first and second ends, the first end lying approximately on the axis of rotation of said separation column; and

a polytetrafluoroethylene collar disposed about and extending past said first end.

7. A method of countercurrent chromatography comprising:

filling a separation column through a feed tube with a first solvent, said separation column having an axis of rotation perpendicular to a main axis of revolution, the feed tube being located coaxial with the main axis of rotation;

centrifuging said filled separation column by revolving said filled separation column in a plane perpendicular to the main axis of revolution, the main axis of revolution lying outside said separation column, at various predetermined angular velocities and in a predetermined direction while simultaneously rotating said separation column about its own axis of rotation at the same angular velocity as the revolution of said separation column and in a direction whereby the feed tube will not twist;

introducing a sample solute to be separated into the moving separation column;

pumping a second solvent, immmiscible with said first solvent, into the moving separation column; and

recovering the separating solute fractions leaving the separation column.

8. The method of claim **7** wherein said first solvent is heavier than said second solvent.

9. The method of claim **7** wherein said first solvent is lighter than said second solvent.

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