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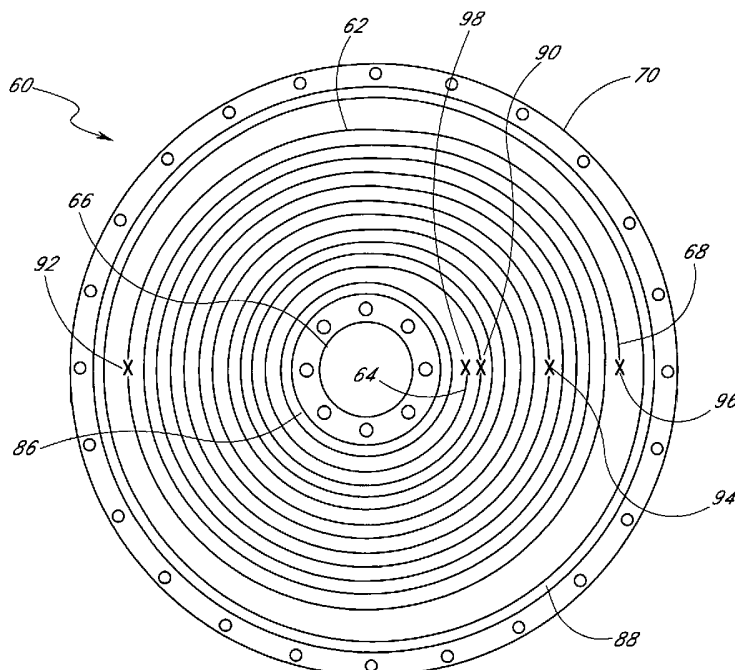
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60/464,665 21 April 2003 (21.04.2003) US</p> <p>(71) Applicant (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health, Office of Technology Transfer, 6011 Executive Blvd., Suite 325, Rockville, MD 20852 (US).</p> <p>(72) Inventor; and</p> <p>(75) Inventor/Applicant (for US only): ITO, Yoichiro [US/US]; 6003 Melvern Drive, Bethesda, MD 20817 (US).</p> | <p>(74) Agent: ALTMAN, Daniel, E.; Knobbe, Martens, Olson And Bear, LLP, 2040 Main Street, Fourteenth Floor, Irvine, CA 92614 (US).</p> <p>(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.</p> <p>(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).</p> |
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- (54) Title:** SPIRAL COLUMN FOR DUAL COUNTERCURRENT CHROMATOGRAPHY



- (57) Abstract:** A plate apparatus for use in dual countercurrent chromatography comprises a disk shaped column support having a spiral groove formed on its surface. Preferably, at least one layer of fluid flow tubing is positioned substantially within the spiral groove. In some embodiments, the dual countercurrent chromatography effect is produced by rotating the disk shaped column on a planetary motion device.



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SPIRAL COLUMN FOR DUAL COUNTERCURRENT CHROMATOGRAPHY

Background of the Invention

Field of the Invention

[0001] The invention relates to dual countercurrent chromatography systems, and more particularly to an improved instrument design for use in dual countercurrent chromatography.

Description of the Related Art

[0002] Chromatography is a separation process that is achieved by distributing the substances to be separated between a mobile phase and a stationary phase. Those substances distributed preferentially in the moving phase pass through the chromatographic system faster than those that are distributed preferentially in the stationary phase. As a consequence, the substances are eluted from the column in inverse order of their distribution coefficients with respect to the stationary phase.

[0003] Chromatography is widely used for the separation, identification, and determination of the chemical components in a complex mixture. Chromatographic separation can be utilized to separate gases, volatile substances, nonvolatile material, polymeric material, and a wide variety of organic and biological substances.

[0004] The performance of countercurrent chromatography (CCC) depends largely on the amount of stationary phase retained in the column, which determines both the resolving power of the solute peaks and the sample loading capacity. Numerous CCC systems have been developed to optimize the retention of the stationary phase of a sample in the column. The maximum attainable retention level tends to fall sharply with the application of higher flow rates of the mobile phase, resulting in loss of peak resolution. Consequently, the applicable flow rate has become one of the major limiting factors in CCC.

[0005] Some CCC systems utilize a complex hydrodynamic motion in two solvent phases within a column comprising a rotating coiled tube. If, for example, a horizontally mounted coil is filled with water and is rotated around its own axis, objects present in the column that are heavier than water will tend to move toward one end of the coil

while objects lighter than water will tend to move toward the other end. In this way, one end of the coil can be called the "head" and the other end, the "tail" of the coil. When the coil is filled with two immiscible solvent phases, the rotation establishes a hydrodynamic equilibrium between the two solvent phases, where the two phases are distributed in each turn at a given volume ratio (equilibrium volume ratio) and any excess of either phase remains at the respective tail of the coil for each solvent.

[0006] When the coil is filled with one of the solvents and the other solvent is eluted from the coil from its head end, the hydrodynamic equilibrium tends to maintain the original equilibrium volume ratio of the two phases in the coil and thereby a certain volume of the other phase is permanently retained in the coil while the two phases are undergoing vigorous agitation with rotation of the coil. As a result, the sample solutes present in one phase and introduced locally at the inlet of the coil are subjected to an efficient partition process between the two phases and are chromatographically separated according to their partition coefficients. In the most advanced CCC technique called high-speed CCC, the unit gravity is substituted with a centrifugal force by applying planetary motion to the coiled column.

[0007] In some cases, CCC utilizes a multi-layer coil as a separation column to produce a high efficiency separation with relatively favorable retention of the stationary phase in many solvent systems. Thus, CCC has been employed to achieve efficient separation of compounds in a sample solution under relatively high flow rates.

[0008] Many previous column designs have relied on the use of a helical coil of tubing. U.S. Patent No. 4,430,216, hereby incorporated by reference in its entirety, describes a preparative CCC utilizing a multiple layer coiled column. The coiled column design includes a length of plastic tubing wound around a coil holder to form multiple layers of the coil. Although this system works reasonably well for some solvents, these systems often fail to retain a satisfactory amount of the stationary phase for highly viscous, low interfacial solvent systems such as polymer phase systems, which are useful for the separation of macromolecules and particulates. In addition, the coiled tubing configuration is difficult to assemble, and connecting the ends of neighboring spiral tubing is rather difficult.

[0009] Another structure that can be used in a CCC column assembly comprises a plurality of separation disks having a plurality of spiral flow channels carved, etched, or molded on the surface of a first side of each separation disk as described in U.S. Patent Number 6,379,973, for example, which is hereby incorporated by reference in its entirety. The spiral flow channel has an inlet end and an outlet end, wherein fluid typically flows along the path of the spiral channel from the inlet end to the outlet end. The spiral channel of one separation disk can be serially connected to the spiral channel of another separation disk by stacking multiple separation disks adjacent to one another with a septum separating each pair. Preferably, an outlet end of a channel on one disk connects to the inlet end of the channel on the next adjacent disk.

[0010] Another design, which allows two mobile phases to simultaneously elute through a column in opposite directions is known as dual countercurrent chromatography (dual CCC). A column useful for dual countercurrent foam separation is described in U.S. Patent No. 4,615,805, which is hereby incorporated by reference in its entirety. Such a design is depicted in FIG. 1A. Dual CCC is capable of genuine “countercurrent” chromatography as lighter and heavier phases move in opposite directions to each other through the column. Dual CCC designs typically feature a coiled column architecture with five flow channels serving the column: two inlets 261, 262 at opposite ends of the column, two outlets 265, 267 at opposite ends of the column, and a sample injection channel 270 in the middle of the column. Although dual CCC methodologies are generally more complicated than ordinary CCC, dual CCC has certain advantages. In dual CCC, solutes with very high or low K values elute out quickly while those with K values around 1 can remain almost permanently in the column. Further, dual CCC allows both liquid-liquid CCC and liquid-gas CCC (foam CCC).

[0011] Existing designs for dual CCC typically feature a number of problems. For example, it can be difficult and time consuming to bring a column to hydrodynamic equilibrium. Further, feed tube inserts and T-connectors can interfere with the continuity of the mobile phases and the overall performance of the column.

Summary of the Invention

[0012] One aspect of the invention is a column support for use in a dual countercurrent chromatography apparatus, including: a surface, a spiral groove formed in the surface, a first inlet connected to the spiral groove, a first outlet connected to the spiral groove such that the first inlet and the first outlet are separated by a portion of the spiral groove, a second inlet connected to the spiral groove, and a second outlet connected to said spiral groove such that the second inlet and the second outlet are separated by a portion of the spiral groove.

[0013] Another aspect of the invention is a column support for use in a dual countercurrent chromatography apparatus, including: a surface, a spiral groove formed in the surface, a column tubing positioned substantially within the spiral groove, a first inlet connected to the column tubing, a first outlet connected to the column tubing such that the first inlet and the first outlet are separated by a portion of the column tubing, a second inlet connected to the column tubing, and a second outlet connected to the column tubing such that the second inlet and the second outlet are separated by a portion of the column tubing.

[0014] A further aspect of the invention is a method of performing a dual countercurrent chromatography separation including: introducing a first mobile phase into a spiral groove in a disk, introducing a second mobile phase into the spiral groove, and energizing the disk so that the first mobile phase moves in the spiral groove toward a head end and the second mobile phase moves in the spiral groove toward a tail end.

Brief Description of the Drawings

[0015] FIG. 1A is a cross-sectional view of a prior art dual CCC apparatus.

[0016] FIG. 1B is a cross-sectional view of a dual CCC column illustrating current flow.

[0017] FIG. 2A is a plan view of a spiral disk column support for a dual CCC apparatus.

[0018] FIG. 2B is a cross-sectional view of a spiral disk column support for a dual CCC apparatus.

[0019] FIG. 3 is a plan view of a spiral column support for a dual CCC apparatus.

[0020] FIG. 4 is a cross-sectional view of a spiral disk dual CCC apparatus that includes a planetary motion apparatus.

Detailed Description of the Preferred Embodiment

[0021] Embodiments of the invention will now be described with reference to the accompanying Figures, wherein like numerals refer to like elements throughout. The terminology used in the description presented herein is not intended to be interpreted in any limited or restrictive manner, simply because it is being utilized in conjunction with a detailed description of certain specific embodiments of the invention. Furthermore, embodiments of the invention may include several novel features, no single one of which is solely responsible for its desirable attributes or which is essential to practicing the inventions herein described.

[0022] Although embodiments of the invention have various applications, many advantageous embodiments of the present invention are directed to an improved plate apparatus for use in dual countercurrent chromatography. Applicable chromatography techniques include those using synchronous planetary motion such as X-type, J-type, and I-type chromatography. The apparatus and methods described herein can also be applied to high-speed countercurrent chromatography (HSCCC) with high flow rates. The plate design may also be employed in large column applications for industrial-scale separations of samples by mounting the column assembly on a slowly rotating horizontal shaft.

[0023] Some aspects of the invention are based, in part, on the fact that system performance is improved when the column used in dual counter current chromatography utilizes a spiral configuration embodied in one or more grooves in a plate or disk, also referred to herein as a "column support." References to a "column" in this context can mean that the spiral groove itself functions as a column by constraining one or more mobile phases that participate in the chromatographic separation. In some other embodiments, however, a length of tubing such as PTFE can be inserted in the spiral groove such that the tubing takes on the spiral shape of the spiral groove and then functions as the column that physically constrains one or more mobile phases.

[0024] In embodiments in which the spiral groove itself functions as a column, it is advantageous to seal the open side of the groove with a Teflon® sheet or other suitable

material, effectively creating an enclosed spiral column. It is preferable that the enclosure be robust enough to withstand the force of a mobile phase when the column rotates and thereby be able to maintain the structural integrity of the column during operation. This type of column construction is most suitable for a small capacity dual CCC column.

[0025] In embodiments in which column tubing is placed inside the groove, it is advantageous to use tubing that is approximately the same size as the groove, or slightly narrower than the width of the groove to prevent the tubing from sliding during use. Tubing made from polytetrafluorethylene (Teflon®) or polychlorotrifluoroethylene (Kel-F®) has been found suitable for use with the present invention. Further, 1.6 mm to 2.6 mm internal diameter tubing has been found suitable for use with the present invention. Column construction which features column tubing positioned within the groove is most suitable for a large capacity dual CCC column.

[0026] As indicated above, FIG. 1A depicts a dual CCC apparatus as disclosed in U.S. Pat. No. 4,615,805. The loops depicted in FIG. 1A represent a coiled column design which is configured to operate on a planetary motion device.

[0027] FIG. 1B depicts a functional schematic of a dual CCC column. Although dual CCC columns are rarely linear, this example is depicted as linear to illustrate the relative positions of inlets and outlets, as well as the directions of current flow.

[0028] In FIG. 1B, a first inlet 90 introduces a first mobile phase into the column. The first mobile phase traverses most of the length of the column and exits the column at a first outlet 96. At the same time, a second inlet 92 introduces a second mobile phase to the column. The second mobile phase traverses most of the column in the opposite direction of the first mobile phase. The second mobile phase exits the column at the second outlet 98. At some point along the length of the column, typically near the middle, a sample feed channel 94 introduces a sample to the column. Typically, a sample will contain a plurality of components having different physical and/or chemical properties and it will be advantageous to separate them. When the column is in use, the various components will preferentially partition between the phases, and move along with one or the other mobile phases depending on the partition coefficient. Components may separate based on hydrophobicity, solubility, or any number of other properties. Various types of collector molecules which have affinity

for particular analytes are known in the art and may be used in conjunction with the present invention.

[0029] As the mobile phases exit the column at their respective outlets, some of the components from the sample can be collected at those outlets over a period of time. Some components may remain in the column and be extracted later by some other method.

[0030] A dual countercurrent chromatography apparatus accordingly to the present invention can be used in a variety of applications. Accordingly, the manner in which components of the sample are recovered will typically depend on the particular application.

[0031] The invention can be used to purify samples for further analysis, such as mass spectra analysis. The invention can be used in the analysis of agrochemicals such as carbaryl, fenobucarb, or methomyl in vegetable oil. The present invention is particularly useful in this way as dual CCC has an advantage over standard CCC techniques in that both hydrophilic and hydrophobic components can be washed out of a column simultaneously. Further, dual CCC can overcome the problem of loss of the stationary phase due to multiple sample injection. Accordingly, repetitive sample injection can be performed with high reproducibility and with minimal contamination from the components retained in the column.

[0032] In some embodiments, the present invention can be used in the analysis of trace pollutants in a liquid, such as water. For the analysis of a minute component in a large bulk of liquid, it is advantageous to adjust the partition coefficient of the target compound to near one. This allows continuous feeding of the sample solution into the column through one of the solvent feed lines to retain the target compounds in the column for a long period of time. The target compounds can later be recovered through the sample feed line or directly from the column in a concentrated state.

[0033] Some embodiments feature foam separation using a surfactant. Preferably, an anionic or cationic surfactant is used to facilitate the isolation of counter-ions in foam fractions at a highly concentrated state.

[0034] Some embodiments feature foam separation without using a surfactant. For example, water and nitrogen gas can be used in a dual CCC separation. The foam producing components can be collected in the foam fraction. This technique is particularly useful in isolating some peptides, such as bacitracin components.

[0035] It has been discovered that the use of a spiral channeled disk design instead of a coiled tubing design in constructing a dual CCC column presents numerous benefits. Accordingly, some embodiments of the present invention include a dual CCC apparatus using a spiral disk column. In preferred embodiments, one or more spiral flow channels are carved, etched, or molded into a disk to create a column. Some embodiments further include the use of one or more layers of column tubing to line a flow channel such that fluid can flow through the column tubing. Whether the column is formed by a groove or by tubing in a groove, a plurality of flow tubes can be connected to the column to add or remove solvents, gases, samples, reagents, and/or combinations of these.

[0036] FIGS. 2A and 2B depict a plan view and cross-sectional view respectively for a spiral groove disk design on a column support 60. Here, a spiral groove 62 is depicted which leads outwardly from an inner end 64 proximal to a center opening 66 of the column support 60, and ends at an outer end 68, proximal to the outer rim 70 of the column support 60. In this embodiment, the inner end 64 and outer end 68 are located at substantially the same angular position on the column support 60.

[0037] Although the spiral groove 62 is illustrated here as having a rectangular cross-section, it will be appreciated that grooves having other regular or irregular shapes as cross-sections can be used. In some preferred embodiments, for example, a groove having an arcuate or semi-circular cross-section can be used. Multiple interleaved grooves on a single column support are also contemplated for use in some embodiments of the present invention. Those of skill in the art will appreciate that the dimensions, shape, number of turns, and number of grooves can vary substantially and will generally be determined by the various requirements and constraints of a particular chromatography application. Similarly, the decision whether to line the groove with a length of column tubing will depend on the chromatography application. In some embodiments, one spiral groove is used which is approximately 1 mm wide and 2 mm deep, while the ridge defining the groove is approximately 2 mm wide (so as to create a pitch of 3 mm).

[0038] FIG. 2A also depicts two O-rings. An inner O-ring 86 and an outer O-ring 88 can be used to seal the column assembly from the environment when the column is in use.

Preferred techniques for using the column are discussed *infra* with regard to the operation of a column on a planetary motion apparatus.

[0039] In FIG. 2A, each “X” on the plan view represents the location of a channel connection point where an inlet or outlet tube can be connected. This particular embodiment features five such flow channels, two inlets, two outlets, and one sample feed channel. Although the following discussion is directed primarily to embodiments which utilize a length of tubing as a column, similar flow channels can be used in embodiments in which the spiral groove itself acts as the chromatography column.

[0040] A first inlet 90 is located near inner end 64 while a corresponding first outlet 96 is located near the outer end 68. A second inlet 92 is located near the outer end 68 while its corresponding second outlet 98 is located near the inner end 64. As depicted in FIG. 2A, the second inlet 92 is not spatially proximal to the outer end 68 since the two points are on substantially opposite ends of the column support 60. The points are termed “near,” however, since they are relatively close to one another from the perspective of a mobile phase traversing the entire path of the spiral column 62. In this way, it should be noted that the physical positions of inlets and outlets should be considered primarily with respect to the overall path of the spiral groove, or column tubing within the spiral groove. They may be spatially near or far from one another on the actual disk surface as the user sees fit. However, it is preferable, that with respect to the column, the two outlets are located at opposite ends and the two inlets are located somewhat inward from the outlets. Such positioning is advantageous to minimize the loss of a mobile phase before it traverses the length of the column. For example, performance of the column would generally be hindered if the first mobile phase, introduced at the first inlet 90, were to exit at the adjacent second outlet 98, rather than travelling the length of the column to exit at the first outlet 96.

[0041] In this way, the spiral groove 62 physically separates each corresponding inlet/outlet pair such that a mobile phase moving from the first inlet 90 to the first outlet 96 or from the second inlet 92 to the second outlet 98 would have to traverse a substantial portion of the length of a column defined by the spiral groove 62. Accordingly, it is advantageous that each inlet/outlet pair carry a different mobile phase. Note that FIG.s 1B and 2A illustrate the various inlets and outlets using the same reference numerals. Although

the column of FIG. 2A has a spiral configuration, the relationship of the various inlets and outlets can be understood with respect to FIG. 1B. In each case, the columns receive two mobile phases which are made to flow in opposite directions and thereby flow past one another on their respective paths from inlet to outlet, thereby creating a dual countercurrent effect, with the inlet for one mobile phase being located inward along the channel from the outlet for the other mobile phase.

[0042] At some point roughly midway between the inner end 64 and the outer end 68 of the spiral groove 62 is a sample feed channel 94. The sample feed channel 94 can serve as an entry point to the column in the spiral groove 62 for a sample being interrogated. Accordingly, when such a sample is injected into the column, the constituents of the sample can separate. Depending on the actual constituents of the sample, solutes having very high K values will elute quickly in one direction while those with very low K values will elute quickly in the opposite direction. Solute having more moderate K values will be retained longer, producing the desired chromatographic effect. In practicing the present invention, the point at which the sample feed channel enters the column can also be adjusted in one direction or the other as may be desired for any particular chromatography application.

[0043] FIG. 3 depicts a plan view of a spiral column support 60 and shows cross-section views for inlets 90/92, outlets 96/98, and a sample feed channel 94. This particular embodiment features a length of tubing positioned within the spiral groove 62. As shown here, the two inlets 90/92 and the sample feed channel 94 each utilize a capillary tube that enters the column and is perpendicular to the column tube. The two outlets 96 and 98, on the other hand, each utilize a capillary tube that is coupled in a parallel orientation to one end of the column tube. These connector designs are advantageous as they can allow the column to retain a substantially smooth and continuous shape which minimizes turbulence and disruption of flow. Accordingly, preferred designs improve the separation and resolution capabilities of the column when performing chromatography.

[0044] FIG. 4 represents a cross sectional view of a planetary motion device 160 that can be used with a spiral disk column. Here, the column support 60 is shown connected to flow tubes 162 which allow two mobile phases to enter and exit the column through the appropriate inlets 90/92 and outlets 96/98 respectively. A fifth flow tube connects to the

sample feed channel 94 and can be used to introduce a sample being interrogated. In this embodiment, the planetary motion device 160 features the column support 60 functionally connected to a planetary gear assembly 170 that allows the column support 60 to rotate. A sun gear assembly 172 correspondingly allows the column support 60 to revolve around a central axis 176. A counterweight 174 can be used to balance the weight of the column support 60 and the planetary gear assembly 170; the physical motion is provided by a motor 180 in functional contact with the central axis 176. As depicted here, the motor 180 drives a toothed belt 182 which turns a pulley 184 at the central axis 176.

[0045] Other devices which are known in the art and are capable of rotating, revolving, and/or otherwise moving the column support can also be used. An advantageous aspect of the design depicted in FIG. 4, however, is that the revolution motion of the sun gear offsets the rotation motion of the planetary gear such that the flow tubes do not become increasingly twisted with every turn. Appropriate designs for use in connection with the present invention can also include the use of more than one column support on a planetary or other motion device.

[0046] As indicated above, some preferred embodiments of the present invention include a length of column tubing positioned in the spiral groove. By routing fluid, gas, or other mobile phase through tubing arranged on a grooved column support rather than directly inside and in contact with the walls of the channels on a disk, the cost of the disk material and required precision of manufacturing can be significantly reduced. In particular, the disk can comprise any solid material since the disk material itself is not exposed to the mobile phases or the sample. The column support material is advantageously rigid, easily machined or molded, and inexpensive. Examples include polyethylene, polypropylene, and foam plastic. Also, the molding or machining precision required in forming the spiral channel or groove can be reduced where there is no concern regarding fluid leakage or concentration of particles at imperfection points along the groove.

[0047] In addition, leakage can occur in a chromatography apparatus which routes the fluid directly inside and in contact with spiral channels on the surface of a disk when a second disk or other sealing piece is positioned directly above the disk, and must be arranged flush with the channeled surface to avoid leakage of the fluid sample. In addition, when a

column of disks are employed, the fluid connection path between neighboring disks must be precisely aligned such that fluid leakage or stoppage due to complete misalignment is avoided. The use of a single length of tubing for fluid flow avoids these leakage problems because the number of joints or connections present within each spiral and between different spiral layers can be minimized.

[0048] Various types of tubing can be used to form the column within the spiral groove. Tubing with a circular cross section can be used to provide a fluid flow path on the column support along the spiral and radial grooves, however, tubing having a cross-section with a non-circular geometry can provide improved results. For example, tubing with a circular cross section has been found to produce a plug flow in tubing with a small diameter, particularly for the organic mobile phase of a two-phase solvent system with high interfacial tension and/or small density differences between the two phases. Such an effect may be largely reduced by implementing a tubing with a rectangular or triangular cross section. In addition, tubing having a cross section with a rectangular or triangular cross section, for example, can provide improved stacking conditions for implementing multiple levels of spiral fluid flow paths on a single column support. Alternately, tubing having a non-circular cross section can be twisted before positioning on the column support so as to improve the partition efficiency.

[0049] In some advantageous embodiments, a single column support can be configured to accommodate a continuous length of tubing to form several layers of spirals without connection joints, thereby avoiding leakage and contamination of the fluid sample undergoing the chromatography. Thus, in some embodiments of the column support, more than one layer of tubing is positioned in the spiral groove 62. Such a configuration can utilize a multilayer design as disclosed in U.S. Application No. 60/457,058 (filed March 21, 2003, Attorney Docket No. NIH264.001PR) herein expressly incorporated by reference in its entirety.

[0050] The single spiral groove embodiment of the column support 60 provides an asymmetrical distribution of the tubing and/or groove, which may require careful balancing of the column for centrifugation. However, a column support having a plurality of spiral grooves can provide a more advantageous structure which does not require such careful

balancing. In these embodiments, two or more grooves are interleaved and the inlets and outlets are arranged such that the disk as a whole is symmetrical in weight distribution.

[0051] Furthermore, the centrifugal force gradient produced by the spiral pitch in the interleaved groove embodiment of the column support can increase the efficiency of distribution of the heavier phase in the periphery and the lighter phase in the central portion of the column.

[0052] In accordance with the foregoing, certain embodiments of the invention provide an improved plate design for use in dual countercurrent chromatography. It will be appreciated, however, that no matter how detailed the foregoing appears in text, the invention can be practiced in many ways. As is also stated above, it should be noted that the use of particular terminology when describing certain features or aspects of the invention should not be taken to imply that the terminology is being re-defined herein to be restricted to including any specific characteristics of the features or aspects of the invention with which that terminology is associated. The scope of the invention should therefore be construed in accordance with the appended claims and any equivalents thereof.

WHAT IS CLAIMED IS:

1. A column support for use in a dual countercurrent chromatography apparatus, comprising:
 - a surface;
 - a spiral groove formed in said surface;
 - a first inlet connected to said spiral groove;
 - a first outlet connected to said spiral groove such that said first inlet and said first outlet are separated by a portion of the spiral groove;
 - a second inlet connected to said spiral groove; and
 - a second outlet connected to said spiral groove such that said second inlet and said second outlet are separated by a portion of the spiral groove.
2. The column support of Claim 1 wherein the portion of the spiral groove separating the first inlet from the first outlet overlaps with the portion of the spiral groove separating the second inlet from the second outlet.
3. The column support of Claim 1, wherein said column support comprises plastic.
4. The column support of Claim 1, wherein said column support comprises a material chosen from the group consisting of foam plastic, polyethylene, and polypropylene.
5. The column support of Claim 1 further comprising a third inlet connected to said spiral groove.
6. The column support of Claim 5, wherein the third inlet connects to the spiral groove in the portion of the spiral groove separating the first inlet and the first outlet.
7. A column support for use in a dual countercurrent chromatography apparatus, comprising:
 - a surface;
 - a spiral groove formed in said surface;
 - a column tubing positioned substantially within said spiral groove;
 - a first inlet connected to said column tubing;
 - a first outlet connected to said column tubing such that said first inlet and said first outlet are separated by a portion of the column tubing;

a second inlet connected to said column tubing; and

a second outlet connected to said column tubing such that said second inlet and said second outlet are separated by a portion of the column tubing.

8. The column support of Claim 7 wherein the portion of the column tubing separating the first inlet from the first outlet overlaps with the portion of the column tubing separating the second inlet from the second outlet.

9. The column support of Claim 7, wherein said column support comprises plastic.

10. The column support of Claim 7, wherein said column support comprises a material chosen from the group consisting of foam plastic, polyethylene, and polypropylene.

11. The column support of Claim 7 further comprising a third inlet connected to said column tubing.

12. The column support of Claim 11, wherein the third inlet connects to the column tubing in the portion of the column tubing separating the first inlet and the first outlet.

13. The column support of Claim 7, wherein said column tubing has a circular cross-section.

14. The column support of Claim 7, wherein said column tubing has a rectangular cross-section.

15. The column support of Claim 7, wherein said column tubing comprises polytetrafluoroethylene or polychlorotrifluoroethylene.

16. A method of performing a dual countercurrent chromatography separation comprising:

introducing a first mobile phase into a spiral groove in a disk;

introducing a second mobile phase into said spiral groove; and

energizing said disk so that said first mobile phase moves in said spiral groove toward a head end and said second mobile phase moves in said spiral groove toward a tail end.

17. The method of Claim 16 wherein:

introducing the first mobile phase comprises introducing the first mobile phase at a first position on the groove that is separated from both ends of the groove; and

introducing the second mobile phase comprises introducing the second mobile phase at a second position on the groove that is separated from both ends of the groove.

18. The method of Claim 16 wherein:

the spiral groove contains tubing;

introducing the first mobile phase comprises introducing the first mobile phase into the tubing; and

introducing the second mobile phase comprises introducing the second mobile phase into the tubing.

19. The method of Claim 16 wherein said energizing comprises rotation of said disk.

20. The method of Claim 16 wherein said energizing comprises rotation and revolution of said disk using a planetary motion device.

21. The method of Claim 16 wherein said first mobile phase moves past said second mobile phase within the spiral groove.

22. The method of Claim 16 further comprising directing a sample into said spiral groove.

23. The method of Claim 22 further comprising collecting a constituent of said sample.

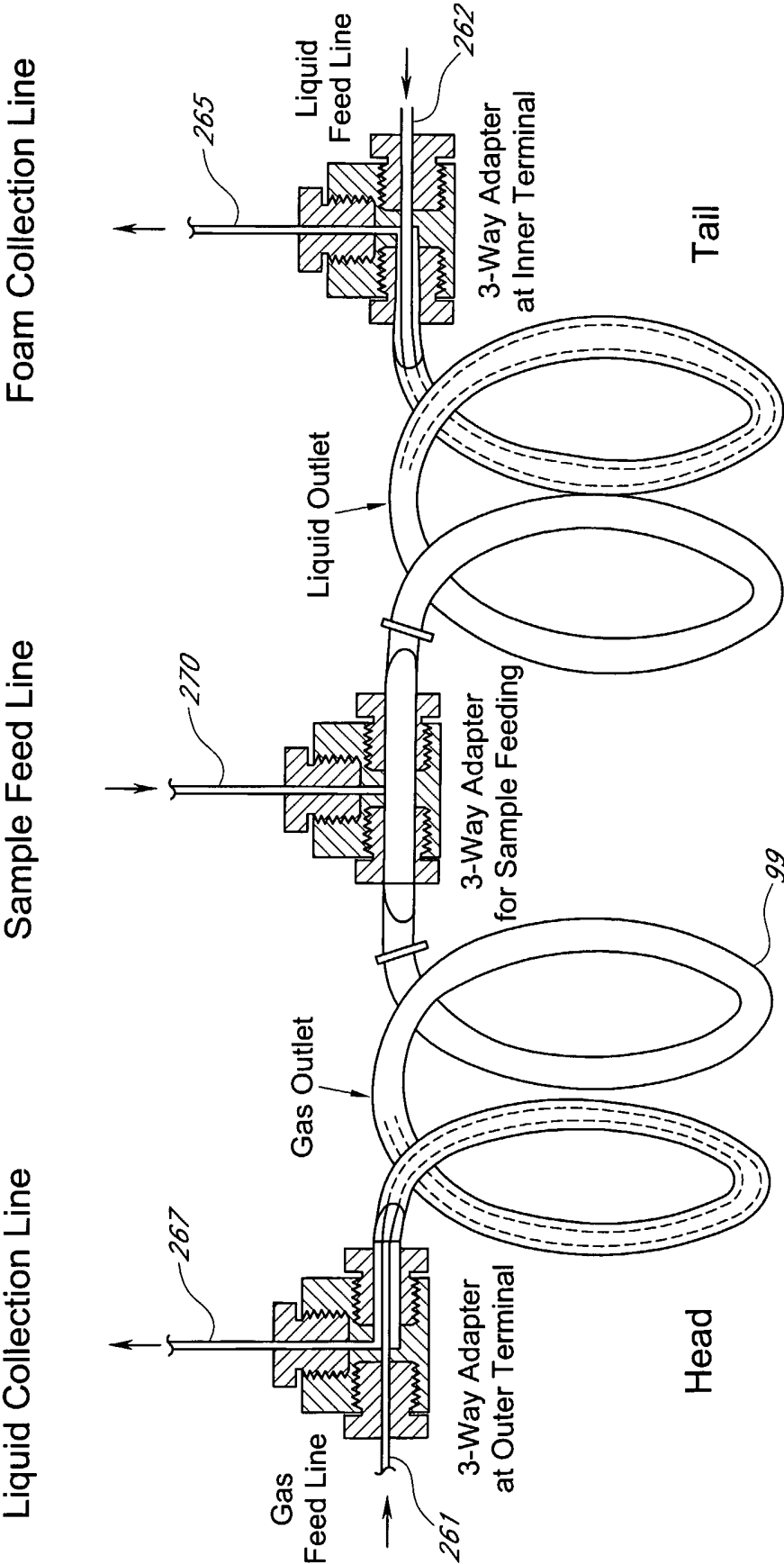


FIG. 1A

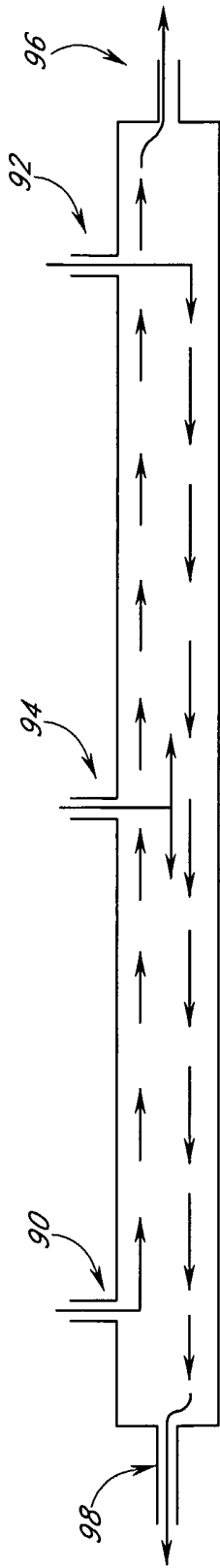
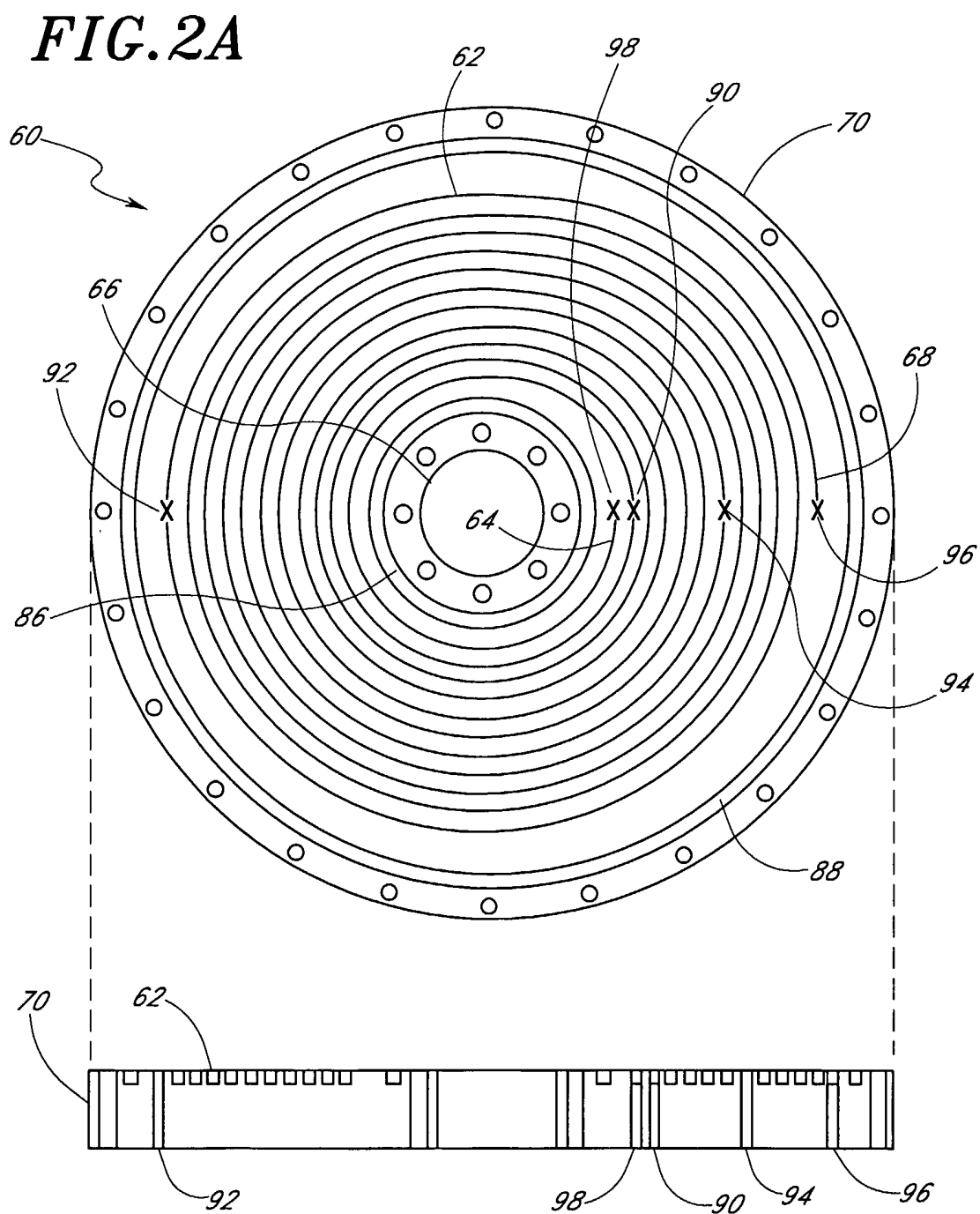


FIG. 1B



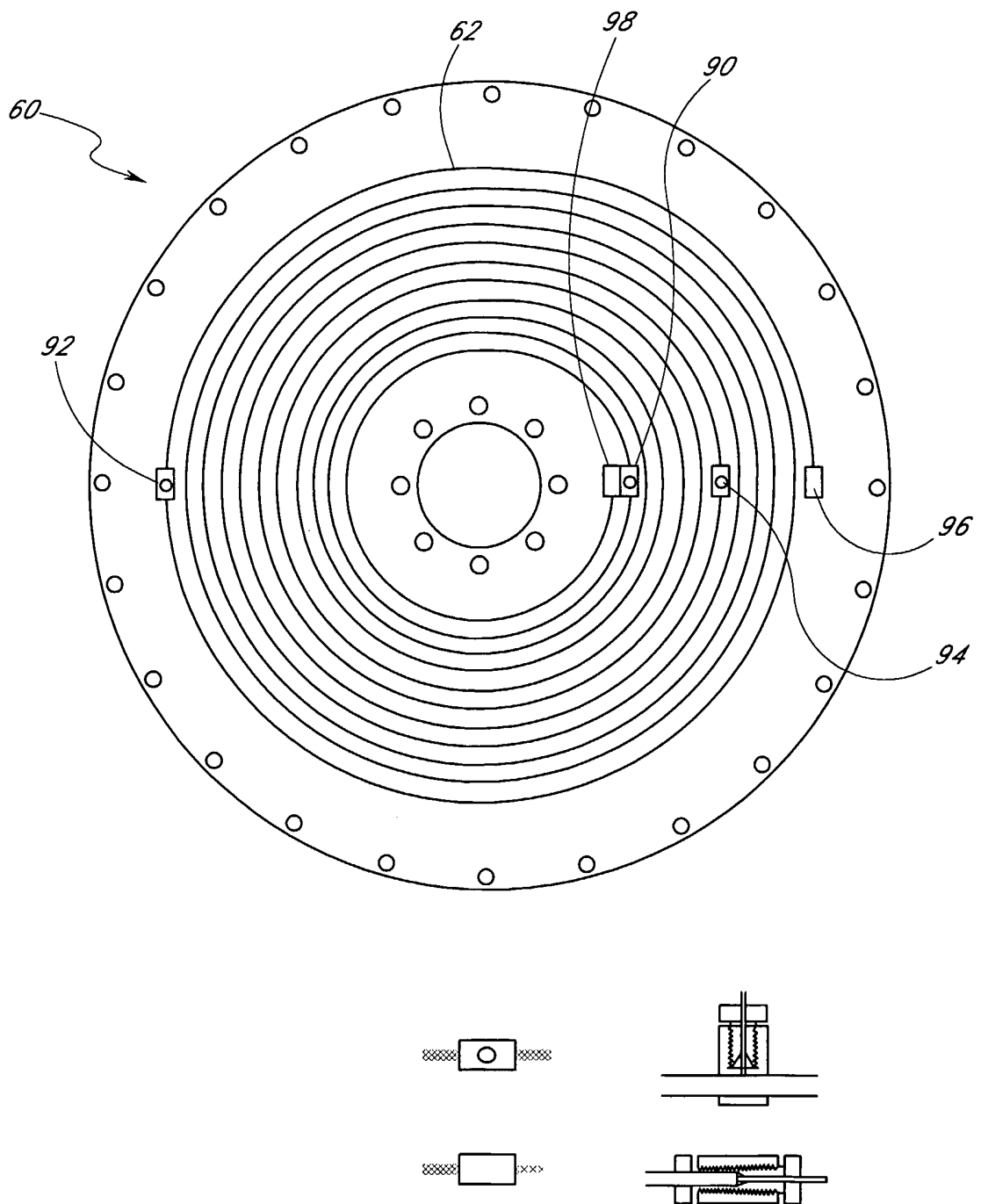


FIG. 3

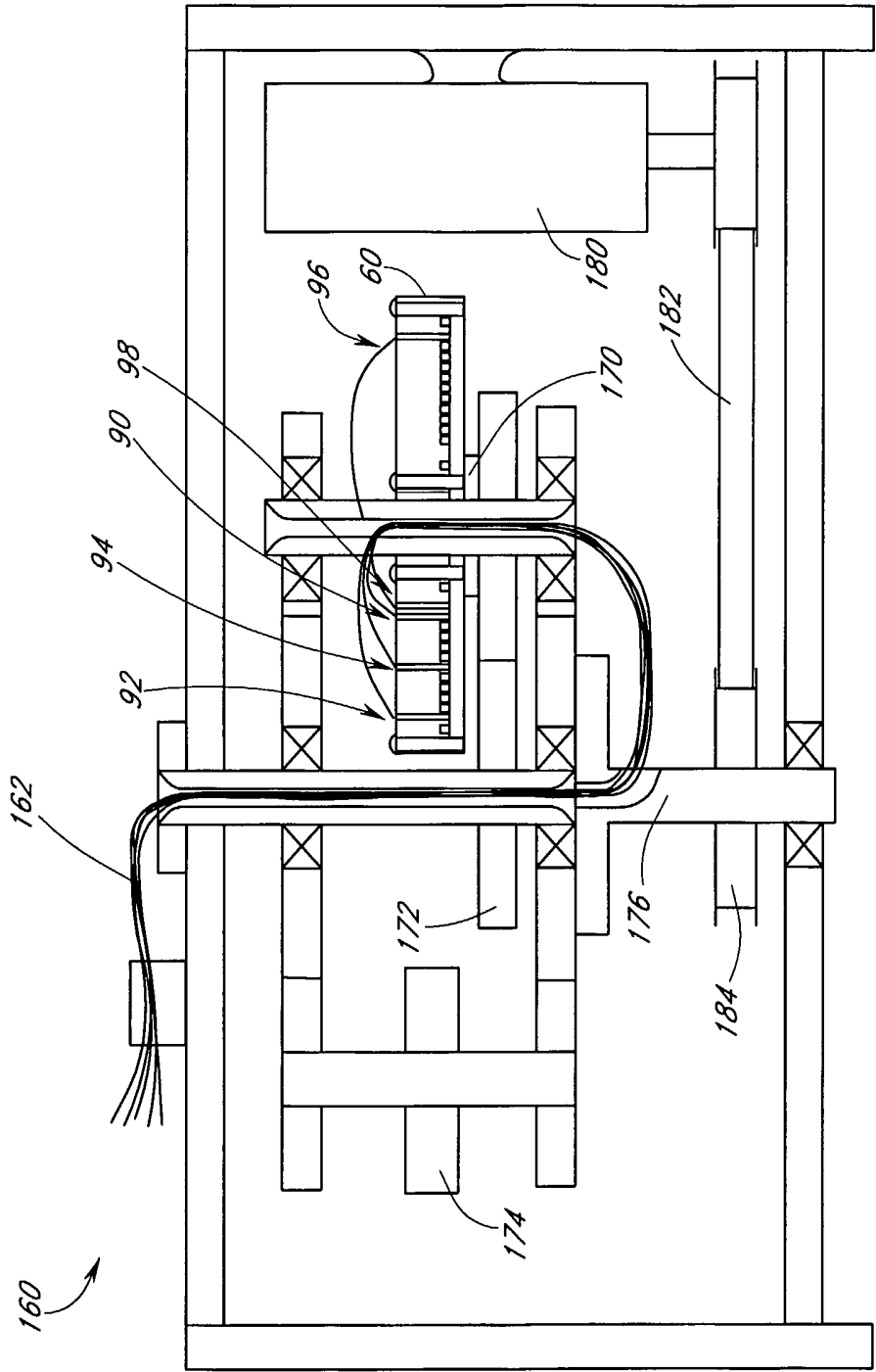


FIG. 4