

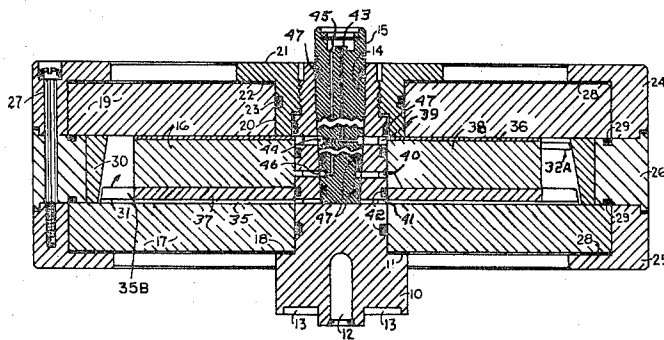
[54] **ROTORS AND ROTOR CORES FOR
CONTINUOUS FLOW CENTRIFUGES**[76] Inventor: **John E. Joyce**, 22 Nelson Rd.,
Weymouth, Mass. 02190[22] Filed: **Aug. 6, 1973**[21] Appl. No.: **385,673****Related U.S. Application Data**[63] Continuation-in-part of Ser. No. 129,055, March 29,
1971, abandoned.[52] U.S. Cl. **233/32, 233/1 A, 233/40,**
233/45, 233/46[51] Int. Cl. **B04b 11/02**[58] Field of Search **233/1 R, 1 A, 21, 27, 28,**
233/32, 33, 44, 45, 46, 40[56] **References Cited****UNITED STATES PATENTS**3,724,748 4/1973 Jacobson 233/21
3,765,601 10/1973 Gulley 233/28**FOREIGN PATENTS OR APPLICATIONS**13,981 5/1890 Great Britain 233/28
57,486 7/1891 Germany 233/27*Primary Examiner*—George H. Krizmanich[57] **ABSTRACT**

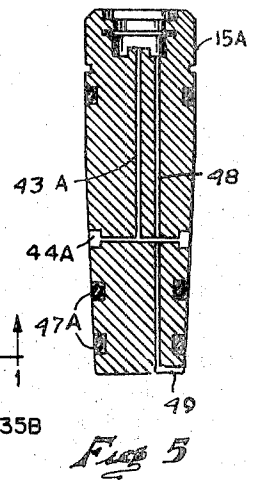
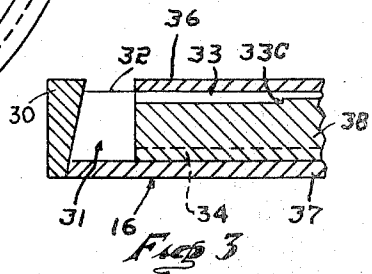
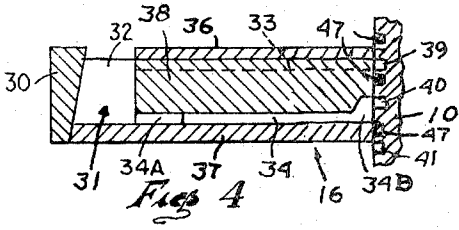
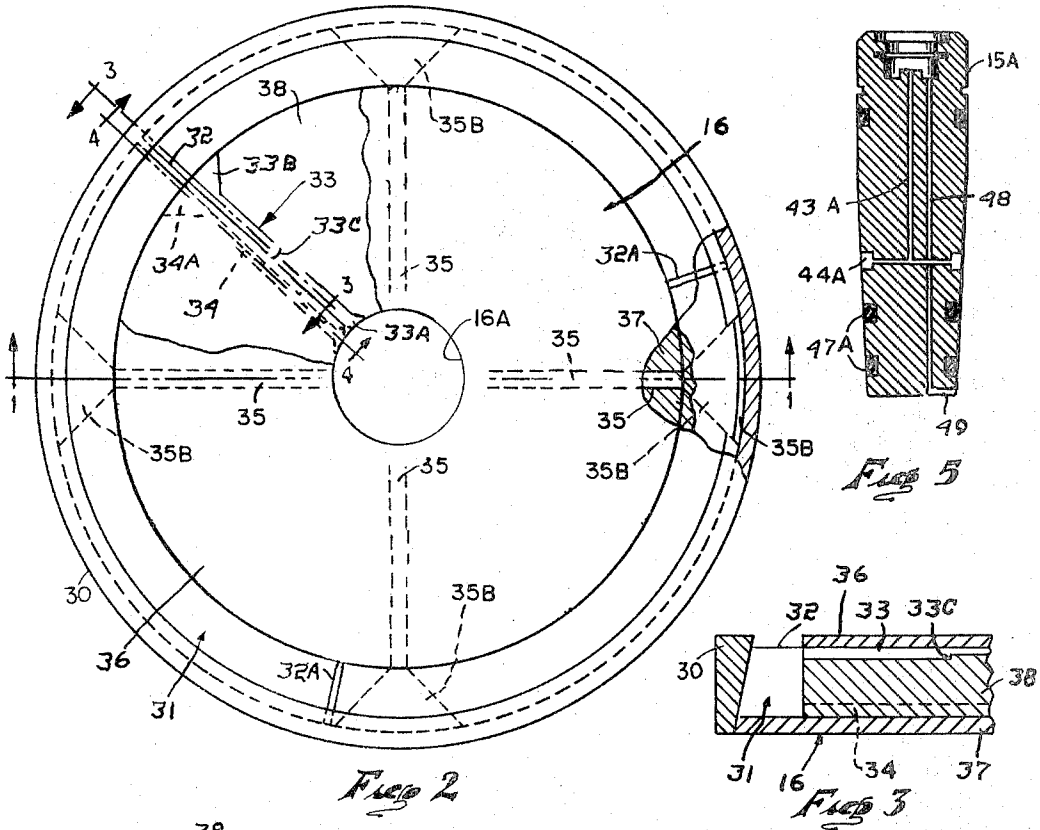
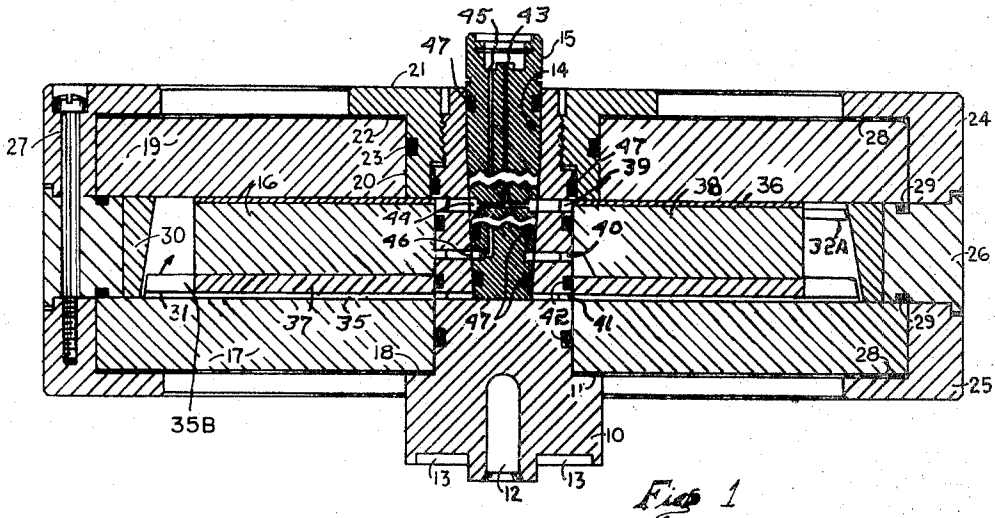
Continuous flow rotors and cores and method of con-

tinuous flow centrifugation therefor are disclosed. In the method, a gradient layer, substantially 360° in extent is formed in a centrifugal field and the sample is made to flow along the inner side of the gradient layer as a concentric band with the relationship between the flow rate of the sample and the volume of its band being such that at the end of the gradient layer, at which the sample is recovered, the sample is substantially spent.

Each rotor has a particle receiving passageway substantially 360° in length with a sample delivery conduit opening into the leading end of the passageway and a conduit for the spent sample leads away from the trailing end thereof. At least one conduit for gradient entry and removal opens into the passageway. The rotors are desirably of the type having removable cores and edge gathering rings and the cores are provided with the conduits and a preferred construction permits a core also to be used if inverted so that it may be used in centrifuges having clockwise drives as well as those in which the rotor is driven counterclockwise.

The present invention relates to methods of continuous flow centrifugation, to rotors for continuous flow centrifuges and to cores to be enclosed in such rotors.

11 Claims, 5 Drawing Figures



ROTORS AND ROTOR CORES FOR CONTINUOUS FLOW CENTRIFUGES

The present application is a continuation-in-part of Ser. No. 129,055, filed Mar. 29, 1971, now abandoned.

BACKGROUND OF THE INVENTION

A continuous flow centrifuge achieves concentration and oftentimes purification of dilute suspensions of many different particles in fluids during the passage of the sample through the spinning rotor.

In some such centrifuges, the only fluid within the rotor at any particular time is the sample and while particle concentrations are achieved, no effective purification of the particles results. In addition, the impingement of sedimenting particles against the boundaries of the rotor chamber is often so harmful to particles of biological origin as to limit their further use.

To overcome these objections, continuous flow rotors are provided that allow two distinguishable and different fluid regions to be present in the rotor at the same time. One region or zone is that of the sample which is continuously flowing from the source with the spent sample, the fluid from which the particles have been removed, being continuously recovered. In another radially outward region or zone, there is a second fluid which stays within the rotor during the treatment of its sample. This zone is usually called the gradient zone and consists of a layer of fluid whose density is greater than that of the sample with its density increasing with the distance from the rotor axis, the density gradient being usually a concentration gradient of a relatively non-diffusing suitable solute such as salt or sucrose. The gradient functions to entrap particles sedimenting from the sample zone without further radial movement at that location where their density is equalled by that of the gradient. The travel of particles of greater or lesser density is varied accordingly. As a consequence, there will be in the gradient, particles separated radially as individual populations of different densities that may be recovered separately after the sample flow has been terminated and while the rotor is spinning. Even in the case of particles that cannot be suspended within the gradient, it functions to so curb their radial velocity that, depending on the flow period, their impingement with the outer boundary of the rotor chamber is prevented even though no effective purification is accomplished.

Zonal rotors have separate sectors and the sample is divided into streams flowing separately through its sectors. Such sectors are of relatively large volume, each sector typically representing 25 percent of the capacity of the rotor. The distribution of the sample to the sectors tends to be unequal with the result that in the case of the sector or sectors receiving the greater volume, the sample is usually not spent when discharged with the result that the subsequent analysis cannot be as accurate as is often desired.

SUMMARY OF THE INVENTION

The general objective of the present invention is to provide that in continuous flow centrifugation, the entire volume of the sample is uniformly treated, an objective attained by providing a zonal rotor with an internal particle collecting passageway or sector that is substantially 360° in extent. A conduit for the sample

opens into the leading end of the passageway and a conduit for the spent sample leads from the trailing end thereof. At least one gradient entry and removal conduit opens into the passageway. In practice, the particle-receiving gradient layer is spaced from the periphery of the core with effective separations resulting because the entire sample is uniformly treated with the gradient layer and the flowing layer of the sample close to but not in excess of 360° in length. The term "spent", as used herein, means that substantially all of the wanted particles have been separated from the sample at the leading end.

While the meaning of the terms "leading" and "trailing" are apparent when used in reference to the opposite sides of the wall in the particle-receiving passageways, their meaning is reversed in discussing the sample flow or the gradient layer, the leading end, in both cases being where the spent sample is recovered as that is the leading end of the flow.

Other objectives of the invention are to provide cores for use with rotors having a core-receiving chamber, the cores having the conduits. A further objective is to provide a core construction that permits a core to be used in a centrifuge having a counterclockwise drive and inverted for use in centrifuges having a clockwise drive.

In the accompanying drawings, there is shown an embodiment of the invention illustrative of these and other of its objectives, novel, features, and advantages.

In the drawings:

FIG. 1 is a vertical section through a continuous flow centrifuge rotor;

FIG. 2 is a plan view of the rotor and its ring;

FIG. 3 is a fragmentary section of the centrifuge rotor taken approximately along the indicated lines 3-3 of FIG. 2;

FIG. 4 is a similar section taken approximately along the indicated lines 4-4 of FIG. 2 but showing part of the arbor; and

FIG. 5 is a vertical section through the distributor used in introducing the gradient into and removing it from the rotor.

The continuous flow centrifuge rotor shown in the drawings has an arbor 10 provided with a bottom shoulder 11 and an axial socket 12 and diametrically opposed recesses 13 in its bottom end for the reception of mating parts of the centrifuge drive, not shown, but providing a counterclockwise rotation of the rotor. An axial bore 14 opens through the upper end of the arbor 10 and has its lower portion downwardly and inwardly tapered to detachably receive either the distributor 15 or the distributor 15A which are both complementally shaped and dimensioned to fit therein. Means, not shown, as forming no part of the present invention, are provided for delivering to and recovering fluids from the rotor through whichever distributor is in service on a continuous basis while the rotor is spinning.

A rotor core, generally indicated at 16 and centrally apertured at 16A to fit the arbor 10 is confined between a bottom plate 17, supported by the shoulder 11 and resting on a gasket 18 and a top plate 19 centrally apertured to receive the hub 20 of a clamping nut 21 threaded on the upper end of the arbor and bearing against an interposed gasket 22. The outer surface of the clamping nut hub 20 has an annular groove for a

sealing ring 23 blocking leakage between the hub 20 and the top plate 19.

The outer edges of the plates 17 and 19 are confined between the clamping rings 24 and 25 joined together through an intermediate ring or spacer 26 by screws 27. Gaskets 28 are interposed between each clamping ring and the appropriate one of the plates. The spacer 26 has annular grooves in its faces for seals 29 blocking outward leakage from the core 16. The spacer 26 confines an edge loading ring 30 whose inner face is shown as upwardly and inwardly inclined.

The core 16 is dimensioned to fit within the rotor chamber wall of which is the ring 30 to provide an annular space generally indicated at 31 between its periphery and the ring 30. The space 31 has a barrier or wall 32, conveniently a part of the core 16, and is the passageway receiving the gradient and the particles sedimenting from the flowing sample. The barrier 32 establishes the leading and trailing ends of the passageway 31.

The core 16 has a sample delivery conduit 33 provided with a laterally flared inlet 33A in its central opening and an elongated outlet 33B opening into the passageway 31 on the trailing side of the wall 32. The conduit 33 is shown as having an intermediate step 33C increasing the cross sectional area of its outer portion. A recovery conduit 34 for the spent sample has its inlet 34A opening into the passageway 31 at the leading side of the wall 32 and its outlet 34B in the central opening of the core. The core 16 also is shown as having four gradient entry and removal conduits 35 spaced 90° apart, each conduit 35 opening into the central opening of the core and ports 35B opening upwardly into the passageway 31 close to the ring 30.

The core 16 is shown as comprising a top circular plate 36, a bottom plate 37 of greater diameter, and an intermediate member 38 to the appropriate faces of which the plates 36 and 37 are secured. The member 38 has a radial channel, shown in solid lines in FIG. 2, on its upper face which is closed by the plate 36 to establish the delivery conduit 33 and its bottom face has a radial channel, shown in dotted lines, which is closed by the bottom plate 37 to establish the recovery conduit 34. The bottom plate 37 has radial channels in its undersurface closed when the core 16 is in the rotor by the bottom rotor plate 17 to establish the gradient entry and removal conduits 35.

The core construction above detailed is well adapted for construction with suitable plastics of which methyl methacrylate is a preferred example and permits assembly by means of appropriate cements.

The bottom plate 37 butts against the ring 30 and both are upwardly and inwardly inclined. As stated, the wall 32 engages the ring 30 to provide a barrier in the passageway 31 and for purposes of stabilization, the core 16 is shown as provided with wall extensions or spacing arms 32A adjacent its upper face, each spaced approximately 120° from the barrier 32 and shaped and dimensioned to butt against the upper part of the ring 30 so that they do not block the passageway 31.

It will be noted that the arbor 10 has vertically spaced, radial ports opening into annular channels 39, 40, and 41 in communication, respectively with the core passages 33, 34, and 35. The arbor 10 is also provided with annular grooves for annular seals 42 above and below each port. As may be seen in FIG. 4, the outlet end 34B of the core passage 34 is vertically enlarged

enabling the arbor channel 40 to be spaced a sufficient distance above the arbor channel 41 to provide space for the subjacent seal 42.

The distributor 15 has an axial passage 43 opening radially into an annular channel 44 and a parallel eccentric passage 45 opening radially into an annular channel 46. The channels 44 and 46 are vertically spaced with the channel 44 in communication with the passage 33 through the channel 39 and the channel 46 in communication with the passage 34 through the channel 40. The distributor 15 has annular grooves for sealing rings 47 on both sides of its channels 44 and 46.

The operating procedure for a centrifuge in accordance with the invention is generally similar to that usually followed in continuous flow centrifugation using a density gradient except that a gradient layer approximately 360° in extent is established and the sample flow is along the entire length of that layer.

The distributor 15A, see FIG. 5, is first used. The distributor 15A is identical to the distributor 15 except that, instead of the passage 45, it has a passage 48 parallel to the axial passage 43A and having a radial port 49. When the distributor 15A is seated in the arbor bore 14, the channel 44A opens into the arbor channel 39 which is placed in communication with the passageway 31 by the conduit 33 and the port 49 is in communication with the arbor channel 41 which is placed in communication with the passageway 31 by the conduit 35 whose port 35B opens upwardly into the passageway 31 adjacent the ring 30, the sealing rings 47A isolating the arbor channel 40. The rotor is first filled with water, the water being pumped in through the passage 43A and the conduit 33 with the gradient passages 35 open to relief through the passage 48.

An exact predetermined volume of the gradient is then introduced into the spinning rotor through the passage 43A and the conduit 33 to provide in the passageway 31 a gradient layer, the volume being such that a desired clearance is established between the periphery of the core member 38 and the light edge of the gradient layer to permit the flow of the sample from end to end of the passageway 31 with the sample being an inner concentric layer. When the sample is flowing through the rotor, the clearance between the periphery of the core member and the light edge of the gradient layer decreases with the particle concentration of the sample.

The distributor 15A is then replaced by the distributor 15 and the sample is then pumped into the rotor through the passage 43 and the conduit 33 so that it flows from end to end of the passageway 31 with the spent sample flowing out through the conduit 34 and the passage 45.

When the sample run has been completed, the particles are recovered. For this operation, the distributor 15A is again used with the preferred procedure being to pump water through the conduit 33 to remove the gradient band or layer through the passages 35, the lighter liquid forcing the heavier liquid body ahead of it radially.

In the case of the present invention, however, the flow of the sample is along a path that is approximately 360° in extent with the gradient.

By way of summary, the passageway 31 receiving the gradient and the flowing sample is the annular space 31 defined by the ring 30, the rotor core member 38, the

exposed portion of the plate 37, and the barrier or wall 32 which is so dimensioned that the passageway 31 is approximately 360° in length. The sample delivery conduit 33 has its outlet 33B opening into the passageway 31 at the trailing side of the wall 32, and the sample recovery conduit 34 has its inlet 34 opening into the passageway 31 at the leading side of the wall 32. The conduits 36 open upwardly into the passageway 31 close to the remote inner edge of the ring 30 and all of the core conduits have ports opening through the axial opening of the core in vertically spaced relationship with respective ones of the arbor ports. With the distributor 15A inserted in the arbor socket, the passage 43A is placed in communication with the core conduit 33 for the admission of water and the selected volume of the gradient with the conduit 48 open and in communication with the passages 35. The core conduit 34 is then blocked. With the distributor 15 in its arbor socket, the passage 43 is placed in communication with the sample inlet conduit 33, the passage 45 is placed in communication with the spent sample outlet conduit 34, and the conduits 35 are blocked.

From the foregoing, it will be apparent that the invention provides, in continuous flow centrifugation, a pathway approximately 360° in extent along which the sample flows substantially as a band to be subjected continuously and uniformly to separating forces, the flow and the shape of the sample fluid being so related that at the leading end of the gradient layer, the sample is substantially spent.

I claim:

1. The method of particle separation that comprises the steps of forming in a centrifugal field a gradient layer substantially 360° in extent of uniform thickness throughout, holding said gradient layer captive in said field, providing said layer with closely spaced but positively separated leading and trailing ends, flowing a sample of a lesser density than the gradient into the field at said trailing end along the inner side of the gradient layer substantially as a concentric band and away from said gradient layer at said leading end, and establishing a relationship between the flow rate of the sample and the volume of its band such that at the leading end of the gradient layer the sample is substantially free of heavy particles with such heavy particles held in said gradient layer but separated therein in accordance with their density.

2. A continuous flow centrifuge rotor including an arcuate passageway for a gradient and a flowing sample, said passageway being approximately 360° in length and having trailing and leading ends that are closely spaced, said rotor including a barrier between said ends and having an inlet conduit for the sample opening into the trailing end of the passageway at the inner side thereof and an outlet conduit for the spent sample opening into the leading end thereof at the inner side of said passageway, said rotor also having at least one conduit for use in gradient removal and opening into said passageway, said rotor having an axial socket, and a distributor removably held in said socket with an end exposed, said distributor having a pair of vertical and horizontally spaced passages opening through said exposed end and having vertically spaced ports opening through the side of the distributor, all of said rotor conduits having ports opening in said socket and vertically spaced so that said sample conduit port and the port of one of said other two rotor conduits are

in communication with appropriate distributor passage ports and the port of the other of said two rotor conduits is blocked by said distributor whereby said rotor may also be used by replacing said distributor with a distributor also having a pair of vertical and horizontally spaced passages having ports opening through the side thereof and vertically spaced so that the port of the rotor sample port and the port of the other of said two rotor conduits are in communication with appropriate distributor ports and the remaining rotor conduit port is blocked by said replacement distributor.

3. The continuous flow centrifuge rotor of claim 2 in which the outer wall of the passageway is inwardly inclined and the gradient conduit opens into the passageway close to the remote side thereof.

4. The continuous flow centrifuge rotor of claim 2 in which the sample inlet conduit is above the sample outlet conduit and the gradient conduit is below the sample outlet conduit and the upper distributor port is located to be in communication with the port of the sample inlet conduit and the lower distributor port is located to be in communication with the sample outlet port, and annular seals carried by the distributor, one between said distributor ports, one above the upper distributor port and one below said lower distributor port but above the gradient conduit port.

5. The continuous flow centrifuge rotor of claim 2 in which the sample inlet conduit is above the sample outlet conduit and the gradient outlet port is below said outlet conduit and the upper distributor port is located to be in communication with the port of the sample inlet conduit and the lower distributor port is located to be in communication with the port of the gradient conduit, and annular seals carried by the distributor, one above the upper distributor port and two above said lower distributor port and spaced one above and one below the sample outlet port.

6. In combination and for use in a continuous flow centrifuge rotor having a circular chamber in which there is an arbor having laterally opening ports vertically spaced from one another, a removable core and an edge loading ring member dimensioned to fit within said chamber, said core including a cylindrical member and an underlying plate of greater diameter and dimensioned to seat on the bottom of the chamber within said ring, said core having an axial opening receiving said arbor, said cylindrical member so dimensioned that its periphery is spaced from the ring member with the inner surface of the ring member, the periphery of the cylindrical member and the portion of the plate between the two members establishing a passageway for a gradient and a flowing sample, one member including a barrier blocking said passageway and establishing leading and trailing passageway ends with the passageway otherwise unobstructed and being substantially 360° in extent, said core member having an inlet conduit for the sample in communication with the inner side of the passageway at said trailing end, an outlet passage for the spent sample in communication with the inner side of the passageway at said leading end and said plate having at least one conduit for use in gradient entry and removal opening into said passageway adjacent said ring member, said core member conduits and said plate conduit having ports opening into said axial opening and being vertically spaced for communication with respective ones of the arbor ports.

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7. The combination of claim 6 in which the member having the barrier establishing the leading and trailing passageway ends is the core member.

8. The combination of claim 6 in which the ring member is inwardly inclined and the gradient port is close to the remote edge of the ring member.

9. The combination of claim 6 in which the outer portion of the sample inlet conduit of the core is substantially greater in cross sectional area than its inner portion.

10. The combination of claim 6 in which the gradient conduit of the core is a channel in the exposed surface of the underlying plate and closed when the core is within the chamber.

11. The combination of claim 6 and a second plate, the cylindrical member is between the plates and the sample inlet and outlet conduits are channels on opposite faces of the cylindrical member each closed by the appropriate one of the plates.

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