March 3, 1970 C. H. CHERVENKA ET AL 3,498,531

CONTINUOUS FLOW ULTRACENTRIFUGE

Filed Feb. 16, 1968 2 Sheets-Sheet 1

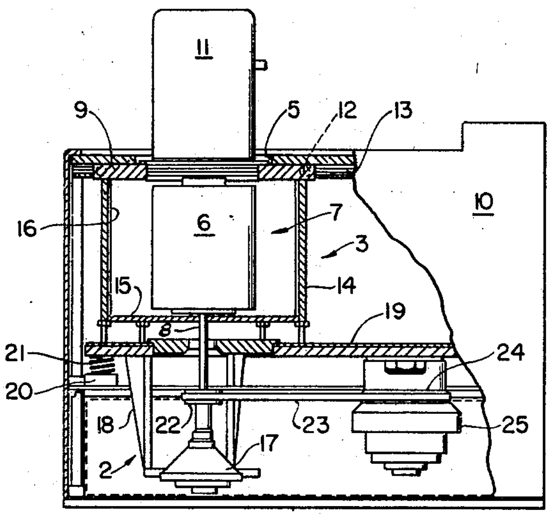

FIG. 1

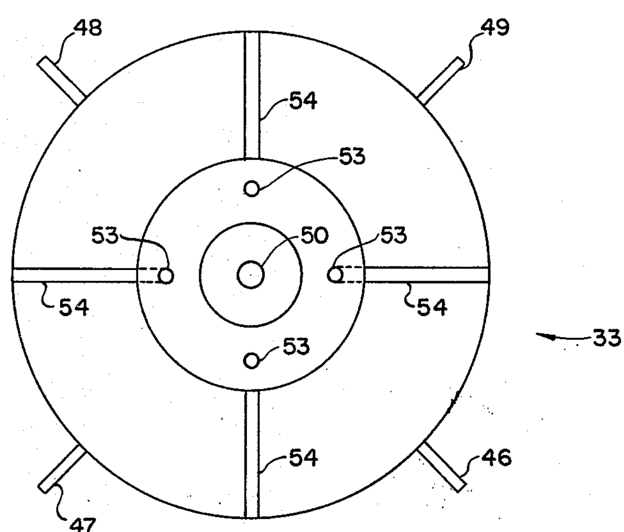

FIG. 4

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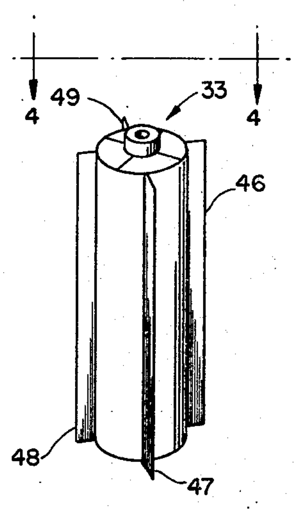

FIG. 3

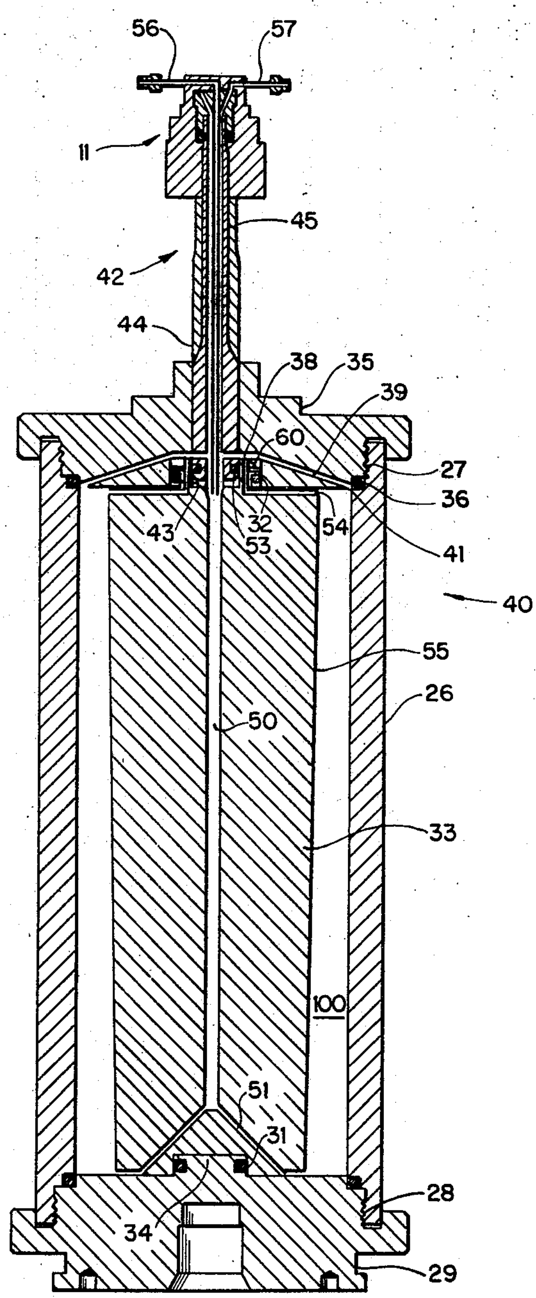

FIG. 2

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CONTINUOUS FLOW ULTRACENTRIFUGE

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Int. Cl. B04b *11/00, 7/04, 1/02*
U.S. Cl. 233—12 5 Claims

ABSTRACT OF THE DISCLOSURE

A predetermined volume of gas, such as air, is introduced into a rotor assembly of a continuous flow centrifuge immediately prior to the introduction of a high density displacement liquid to prevent mixing between such displacing liquid and the density gradient solution containing the centrifuged particles of interest as the displacing liquid forces the density gradient solution from the rotor cavity. The predetermined air volume lodges in a vertical slot provided in the rotor core which slot communicates by way of a connecting radial slot with the outer surface of the core member disposed in the cavity to prevent the displacing liquid from reaching the rotor cavity through this passageway. As a result, all of the displacing liquid enters the rotor cavity at the outside edge of the cavity, thus preventing mixing of the displacthe liquid and the density gradient solution containing the particles of interest.

BACKGROUND

Continuous flow or zonal centrifugation has become an increasingly important analytical tool in the investigation of the nature of numerous biological and other chemical substances. Typically, in continuous flow centrifugation a sample solution is continuously pumped through a rotor cavity which is filled with an appropriate density gradient solution. During centrifugation the particles of interest disperse in a radial direction throughout the density gradient solution and at equilibrium are suspended in the density gradient solution at a location wherein their respective buoyant densities correspond to that of the solution.

To remove the particles of interest the density gradient solution containing the centrifuged particles is displaced from the rotor cavity by means of a liquid having a density greater than the highest density portion of the density gradient solution. Ideally, all of the displacing liquid is fed to the outer edge of the rotor cavity. However, in practice some of the displacing liquid flows through the sample exit passageway provided in the rotor core and enters the rotor cavity at the surface of the rotor core member. Since the lighter density portions of the density gradient solution are located closest to the surface of the core member, mixing between the displacing liquid and the density gradient solution takes place (as the displacing liquid gravitates under centrifugal force toward the outer edge of the rotor cavity) causing substantial dilution of the rotor contents. Such mixing, of course, is highly undesirable and, in fact, limits the usefulness of a continuous flow centrifuge for certain experimental investigations.

One approach to the solution of this mixing problem has been to insert a flexible O-ring in the vertical portion of the sample exit passageway provided in the core, which O-ring acts as a valve to control the flow of fluid through this passageway. The operation of the O-ring valve is governed by the particular rotational speed of the rotor. That is, at low rotational speeds the elastomeric O-ring valve is closed to block the flow of liquids through the sample exit passageway. On the other hand, at high ro-

tational speeds the O-ring valve is subjected to a sufficient centrifugal force to cause the valve to open and allow the flow of fluid through the passageway. Thus, it is apparent that when a flexible O-ring valve is employed the sample solution may be pumped only at rotor rotational speeds above the minimum required to activate the valve and the heavy unloading solution may be pumped only when the rotor is rotated at speeds below this minimum speed. Of course, the particular speed required to activate the O-ring valve may vary with use as the properties of the O-ring change. Moreover, replacement of the O-ring requires the substitution of a second O-ring of very similar properties and size which, from a design standpoint, is extremely difficult to achieve to say the least.

SUMMARY

The present invention contemplates a method for use in a continuous flow ultracentrifuge for preventing the inadvertent mixing of the displacing liquid and the density gradient solution containing the centrifuged particles of interest as the displacing liquid forces the density gradient solution out of the rotor cavity. To this end, a predetermined volume of gas, such as air, is introduced into the rotor assembly immediately prior to the introduction of the heavy density displacing liquid. The predetermined volume of air lodges in a portion of the sample exit passageway provided in the top of the rotor core to block the flow of the displacing liquid through this passageway and, thus, prevents the mixing of the displacing liquid with the density gradient solution contained in the rotor cavity. In an alternative embodiment contemplated by the present invention an additional radial slot is provided in the core member to receive the predetermined volume of air which blocks the flow of the displacing liquid through the sample exit passageway.

Accordingly, a primary object of the present invention is a new and novel method for preventing the inadvertent mixing between the displacing liquid and the density gradient solution contained in the rotor cavity of a continuous flow centrifuge.

Another object is the provision of a method for preventing the inadvertent mixture between the displacing liquid and the density gradient solution contained in the rotor cavity, which method is independent to the rotational speed of the rotor.

Another object is a simple and expedient method of preventing the intermixing of a displacing liquid and a density gradient solution contained in the rotor cavity of a continuous flow centrifuge.

These and other objects and advantages of the present invention will become apparent from the following detailed description when read in conjunction with the accompanying drawings in which:

FIG. 1 is an elevation view, partly in section, showing a continuous flow centrifuge apparatus;

FIG. 2 is an enlarged partial sectional view showing a suitable centrifuge rotor;

FIG. 3 is a reduced schematic perspective view of the rotor core;

FIG. 4 is a plan view of the rotor core taken along lines 4—4 of FIG. 3.

With reference now to the drawings and more particularly to FIG. 1 thereof, it will be observed that the continuous flow ultracentrifuge includes an outer housing **10** which serves to enclose a drive means, designated generally by the reference numeral **2**, and a rotor housing **3**. The top of outer housing **10** is provided with an opening **5** through which a rotor **6** may be passed for mounting within a rotor chamber **7** on a drive shaft or spindle **8**. A movable door **9** threadably receives and supports an upper bearing and seal assembly **11**. Door **9** is provided with spaced rollers **12** which ride in space channels **13** secured to the sides of housing **10** to provide easy access to chamber **7**. A latch mechanism (not shown) cooperates in conjunction with controls (not shown) to releasably lock door **9**.

The side walls of rotor chamber **7** include a generally cylindrical steel member **14** which acts as a guard should the rotor core explode under the strain created by the relatively high rotational velocities at which it is operated. The lower end of member **14** includes a bottom wall **15** likewise made of relative strong material. The interior of rotor chamber **7** may be provided with refrigeration means including evaporator coils **16** which serve to control the temperature therein.

A suitable vacuum seal is formed between bottom wall **15** and spindle **8** as it extends upwardly into chamber **7**. Preferably spindle **8** is made of flexible material and extends downwardly with its lower end journaled in an oil filled bearing assembly **17**. Bearing assembly **17** is supported by space brackets **18** extending downwardly from a resiliently mounted base **19**. Base **19** may be supported from a fixed member **2** by springs **21**. A driven pulley **22** is carried by spindle **8** and is driven by a belt **23** from a drive pulley **24** and motor **25**. Means (not shown) may be disposed in housing **10** for evacuating air from chamber **7**.

The rotor assembly **40**, as best illustrated in FIG. 2, includes a cylindrical bowl **26**, the wall of which is threaded at both ends **27** and **28**. End **28** threadably receives a closure member or base **29** which includes a generally cylindrical well **31** adapted to receive the upper end of drive spindle **8**. Drive spindle **8** fits tightly into well **30** to provide a positive frictional drive connection between spindle **8** and base member **29** whereby rotor bowl **26** may be driven.

The rotor assembly, designated generally by the reference numeral **40**, also includes a rotor core member **33** which fits tightly over a boss **34** provided at the upper end of base **29**. Boss **34** is provided with an O-ring **31** to provide a tight frictional interconnection between core member **33** and base **29** so that the core member **33** rotates with base **29**.

The upper end of bowl **26** threadably receives a cover or lid member **35**. A suitable O-ring **36** is interposed between a shoulder **37** formed on the sides of bowl **26** and the lower edge of lid member **35** while a second O-ring **32** is held in an annular groove formed in the top of core member **33** to provide an effective seal between core **33** and lid member **35**. The lid member **35** is positioned with respect to the rotor core **33** to provide a relatively large annular space **38** between the upper end of the rotor core **33** and the lid member **35**. The lid member **35** is also provided wtih a plurality of radially extending, downwardly sloping bores or holes **39** which communicate between the annular space **38** and the outer edge **41** of the rotor chamber. In practice, four equally spaced holes **39** are formed in lid member **35**. However, it will be appreciated that, due to the nature of the illustrated cross-sectional view, only one such hole (**39**) is shown.

A feed and shaft assembly **42** extends downwardly through an aperture provided in cover member **35** into a well **43** provided in the top of rotor core member **33**. The shaft assembly **42** includes an outer tubular conduit **44** and a concentric inner tubular coduit **45** each of which is adapted to carry fluids into and out of the rotor chamber simultaneously during rotation.

The rotor core **33** is a solid cylinder, formed from a suitable metal, which is tapered slightly in an outward direction from bottom to top and which includes at least four projecting vanes, **46**, **47**, **48** and **49** (best shown in FIG. 3) that divide the rotor cavity into four sector compartments for minimizing turbulence. Core member **33** is provided with a small centrally located axial hole **50** extending from top to the bottom of the core member **33** and which communicates with the rotor cavity **100** by way of a radial extending, downwardly sloping, hole **51** formed in the bottom of the rotor core **33**. In practice, rotor core **33** is provided with four equally spaced holes **51** but, again, due to the nature of the illustrated cross-sectional view, only one hole **51** is shown. The inner fluid-carrying tube **45** communicates with the axial hole formed in the center of the rotor core while the outer fluid-carrying tube **44** cooperates with both the annular space **38** provided at the top of the core **33** and the radial holes **39** formed in the cover member **35** which lead to the outer edge **41** of rotor cavity **100**.

Core member **33** is also provided with four equally spaced vertical extending holes **53** in the top of the core which cooperate with four radially extending slots **54** extending to the surface **55** of core member **33** disposed in rotor cavity **100**. Each radial slot **54** communicates with a vertical hole **53** to form a sample effluent exit passageway.

The inner fluid-carrying tube **45** cooperates with an outlet tube **56** while the outer fluid-carrying tube **44** is associated with a second outlet tube **57**.

To facilitate a complete understanding of the operation of the present invention it is believed it would first be appropriate to discuss briefly the typical operating procedure used in analyzing suitable samples by continuous flow ultracentrifugation techniques. Initially the rotor is loaded by pumping a density gradient solution into the rotor cavity **100** by way of outer tubular line **44**, annular space **38** and downwardly sloping radial hole **39** formed in the cover member **35**. The lightest density portion of the density gradient solution is pumped into the rotor cavity first and enters at the outer edge **41** of the rotor cavity **100**. As successively denser portions of the gradient are pumped into the rotor cavity **100** they in turn force the lighter density portions of the density gradient solutions centripetally toward the surface **55** of the core member **33**. After the gradient is in the rotor cavity, a fluid having a density greater than the highest density portion of the density gradient solution, which fluid is generally referred to as a "cushion," is introduced into the rotor cavity through outer tubular feed-line **44**, annular space **38** and radial holes **39** to completely fill the rotor cavity **100**.

After the rotor cavity has been completely filled with the density gradient solution, the fluid flow through the outer tubular conduit **44** is terminated and the sample solution is introduced into the rotor cavity **100** by way of inner tubular conduit **45**, centrally located axial hole **50** and downwardly sloping radial holes **51** provided in the bottom of core member **33**. The sample solution flows through the rotor cavity **100** from the bottom to the top along the surface of core member **33**, and exits from cavity **100** by way of sample exit passageway comprising radial slot **54**, vertical hole **53**, and annular space **38**. The sample solution then flows out through outer tubular conduit **44** and connecting conduit **56** to a suitable container (not shown).

After centrifugation the density gradient solution containing the centrifuged particles of interest is displaced from the rotor cavity **100** by means of a highly dense liquid. That is to say, a highly dense liquid is fed to the outer edge **41** of the rotor cavity **100** by way of outer tubular conduit **44**, annular space **38**, and radial holes **39**. The displacing liquid being of greater density pushes or forces the lighter density gradient liquid solution containing the particles of interest out of the bottom of the rotor cavity **100** through radial holes **51**, centrally located axial extending hole **50**, inner tubular conduit **45**, and connecting conduit **56** to a suitable fraction collector (not shown). In practice it has been found that some of the displacing liquid flows through the sample exit passageway comprising vertical hole **53** and radial slot **54** provided in the top of the core member **33** and enters the rotor cavity **100** at core surface **55**. As previously discussed, inasmuch as the lighter density portions of the density gradient solution are located toward this surface of the rotor core member, the displacing liquid (being of a much greater density) flows in a radial direction outwardly through the density gradient solution and, thus, intermixes with it. This naturally results in a dilution of the density gradient solution containing the particles of interest and detrimentally affects the end resolution of the ultracentrifugation process.

In accordance with the principles of the present invention, to prevent the intermixing of the displacing liquid and the density gradient solution containing the centrifuged particles of interest, a predetermined volume of gas, such as air, is introduced into the rotor assembly immediately prior to the introduction of the displacing liquid. In particular, the predetermined volume of air is fed by way of outer tubular conduit **44** to the top of the rotor assembly **40**. The volume of air which is introduced through this passageway completely fills each vertical hole **53** provided in the top of core member **33** and partially fills the annular space **38** between cover member **35** and core **33**. It should be noted that the vertical holes **53** are orientated perpendicular to the direction of centrifugal force produced by the rotor assembly and hence the volume of air tends to "lodge" in these vertical holes **53**. The highly dense displacing liquid, which follows the predetermined volume of air down the outer tubular conduit **44**, easily passes through the annular space **38** to the radial holes **39** and enters the rotor cavity **100** at the cavity's outer edge **41**. Of course, since the displacing liquid is of much higher density than that contained in the rotor, its movement to the outer radius is aided by the centrifugal force field. However, the volume of air lodged in the vertical holes **53** blocks the flow of displacing liquid through the sample exit passageways. It should be noted that relatively high pressures would be required to displace the air out of holes **53**, because the only escape passage for this air is by way of radial slots **54**, against the centrifugal field. Consequently, none of the displacing liquid may enter the rotor cavity **100** at core surface **55** and hence intermixing between the displacing liquid and the density gradient solution is prevented. Moreover, the flow of the sample solution in the normal direction (from the bottom to the top of the core) is not hindered in the least since the sample flow rate is sufficient tc sweep the air block out of the sample exit passageway.

The volume of air necessary to effectively block the sample exit passageway while at the same time keeping free the radial holes **39** leading to the outer edge **41** of rotor cavity **100** is generally governed by the particular type of rotor assembly used in the continuous flow centrifuge system. For instance, in a suitable rotor manufactured by the present assignee and designated model B–IX, a volume of around 2.5 to 4.0 milliliters was found to be effective. Of course, it will be appreciated that the necessary volume of air varies according to the circumstances. The volume of air may be conveniently controlled by removing the tubular conduit **57** at the seal housing **11** and flushing it with displacing liquid and then draining the required quantity back out of the line before re-attaching it. The length of empty tubing may then be used to indicate the volume of air contained therein.

In an alternative embodiment commensurate with the principles of the present invention, there is provided in at least one core sector an additional slot or small bore **60** (as indicated by the dotted line) in the top of the core member **33** immediately above the O-ring **32**, which bore extends in a radial direction to interconnect vertical hole **53** and annular space **38**. That is, bore **60** communicates with a small space (not shown) between cover member **35** and the right side of the top of core member **33** to form a generally U-shaped passageway. After forming this passageway, each of the vertical portions **53** of the sample exit passageways is plugged with a suitable stopper (not shown). As a result, the air being fed into the assembly flows through this U-shaped passageway.

In operation the predetermined volume of air introduced into the rotor assembly immediately prior to the displacing liquid lodges in each of the additionally formed bores **60** and thus forces the displacing liquid to flow through radial holes **39** to the outer edge **41** of rotor cavity **100** in a manner previously discussed. It will be appreciated that either one or all four of the sample exit passageways may be modified in this just-described manner if desired.

Numerous modifications and departures from the specific apparatus described herein may be made by those skilled in the art without departing from the inventive concept of the invention. Accordingly, the invention is to be construed as limited only by the spirit and scope of the appended claims.

What is claimed is:

1. A method for use with a continuous flow centrifuge to prevent mixing between the displacing liquid and the density gradient solution containing the centrifuged particles of interest as the displacing liquid forces the density gradient solution from the rotor cavity comprising the steps of introducing a predetermined volume of gas into the rotor assembly and then introducing a displacing liquid into the rotor cavity containing the density gradient solution.

2. A method for use with a continuous flow centrifuge as defined in claim **1** wherein the predetermined volume of gas is transmitted to the entrance to the rotor cavity, said predetermined volume of gas entering the lodging in a portion of a sample exit passageway provided in the top of a rotatable core member disposed in the rotor cavity and displacing liquid is blocked from flowing through the sample exit passageway by the predetermined volume of gas lodged therein and thus forced to flow through an alternate slot communicating with the outer edge of the rotor cavity.

3. In a continuous flow centrifuge apparatus for preventing mixing between displacing liquid and the density gradient solution containing the particles of interest as the displacing liquid forces the density gradient solution from the rotor cavity comprising a generally cylindrical bowl, a cover member secured to the open end of said bowl, the interior surface of said bowl member and said cover member defining a rotor cavity, a rotor core member disposed in said cavity, means for driving said bowl and core members together at a predetermined rotational speed, said core member including a small bore communicating with the bottom portion of said rotor cavity, the top surface of said core member and the interior surface of said cover member defining an annular space, the upper end of said core member including a sample exit passageway connecting said annular space with the edge of said core member, the cover member including a radial extending hole connecting said annular space with the outer edge of said rotor cavity, conduit means passing through an aperture in the cover member, one end of said conduit means communicating with said annular space and means for transmitting a predetermined volume of gas through said conduit means immediately prior to transmitting a displacing liquid through said conduit means, said predetermined volume of gas becoming lodged in said sample exit passageway to block the flow of displacing liquid through the sample passageway and forcing substantially all of the displacing liquid to flow through the radial hole provided in the cover member to the outer edge of said rotor cavity to thereby prevent the intermixing of the displacing liquid and the density gradient solution containing the centrifuged samples of interest.

4. Apparatus as claimed in claim **3** wherein said sample exit passageway includes a vertical orientated portion and a radially extending portion, said predetermined volume of gas being directed to and becoming lodged in said vertical orientated portion.

5. Apparatus as claimed in claim **3** wherein said sample exit passageway includes a vertically orientated portion and a radially extending portion and said core member includes a second passageway including a radially extending portion connecting said vertical portion of said sample exit passageway to said annular space, and means for blocking said vertical portion of said sample exit passageway whereby said predetermined volume of air is directed to and becomes lodged in the radially extending portion of said second passageway.

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WILLIAM I. PRICE, Primary Examiner

U.S. Cl. X.R.

233—16, 28, 46

UNITED STATES PATENT OFFICE

CERTIFICATE OF CORRECTION

Patent No. 3,498,531 March 3, 1970

Charles H. Chervenka et al.

It is certified that error appears in the above identified patent and that said Letters Patent are hereby corrected as shown below:

Column 6, line 28, "the" should read -- and --; line 31, after "and" insert -- the --.

Signed and sealed this 24th day of November 1970.

(SEAL)
Attest:

Edward M. Fletcher, Jr.
Attesting Officer

WILLIAM E. SCHUYLER, JR.
Commissioner of Patents