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APPARATUS AND ARTICLES FOR INCREASING THE RATE OF PARTICLE
SEPARATION AND REMOVAL

3,456,876

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2 Sheets-Sheet 1

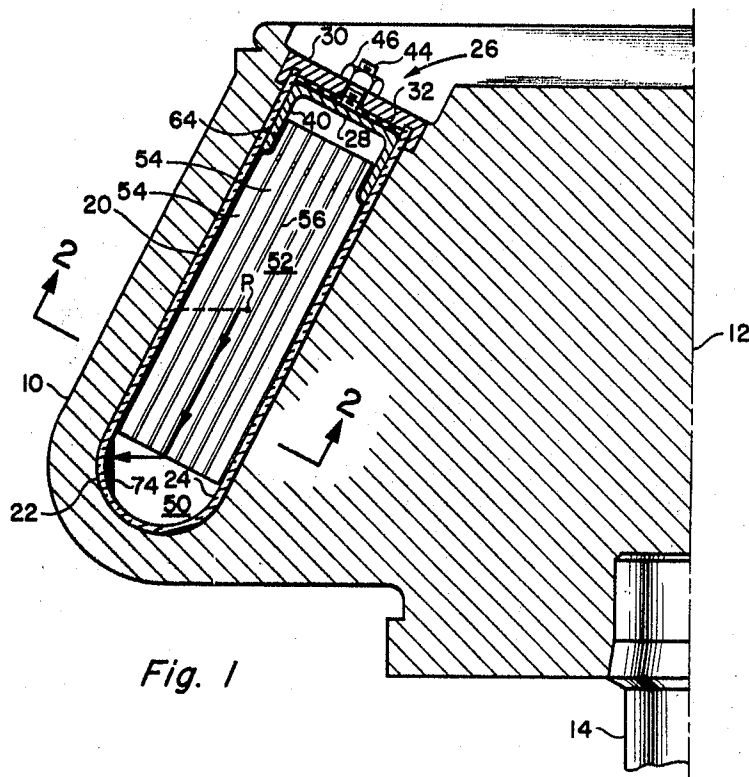


Fig. 1

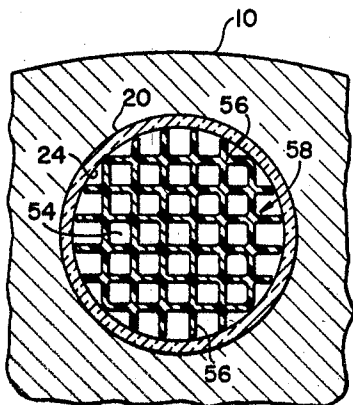


Fig. 2a

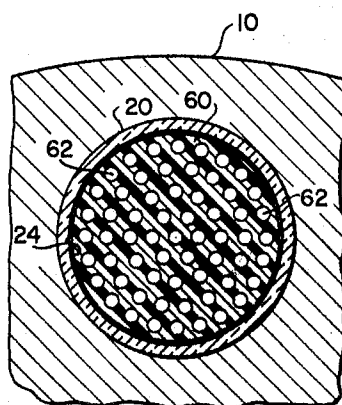


Fig. 2b

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2 Sheets-Sheet 2

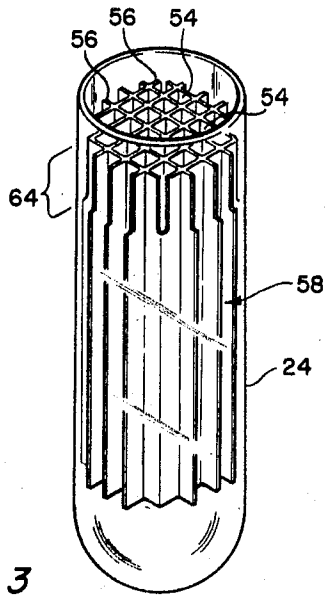


Fig. 3

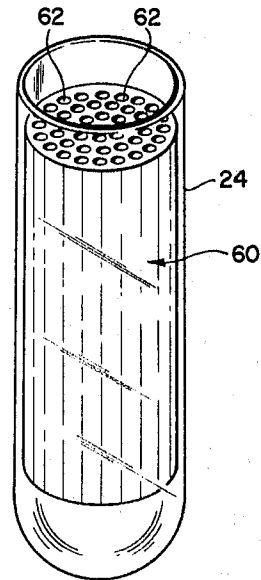


Fig. 4

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APPARATUS AND ARTICLES FOR INCREASING THE RATE OF PARTICLE SEPARATION AND REMOVAL

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4 Claims

ABSTRACT OF THE DISCLOSURE

A receptacle for use with a centrifuge rotor in which the volume of the receptacle includes a particle collection zone and an adjacent zone which in turn is divided into a plurality of small compartments by a multi-cellular insert. The receptacle itself is disposed in the rotor at an angle to the axis of rotation so that each compartment has at least one wall which is inclined at an oblique angle with respect to the direction of centrifugal force. By subdividing the volume inside the receptacle into a number of smaller compartments the mean distance through which the centrifuged particles of interest must travel before precipitating along a centrifugal surface is substantially shortened. This results in an overall reduction of the time required to centrifugally sediment particles from a given volume of solvent.

This invention relates generally to the separation, by centrifugation, of particles dispersed in a medium, and particularly, to apparatus and articles for increasing the rate of separation and removal of the particles from the medium in which they are dispersed without increasing the centrifugal force.

High speed centrifugation is a well-known technique for accomplishing the separation or removal of particles from suspension or solution. (The term "particle," as used throughout this application, is intended to encompass a broad range of particulate matter. At one end of the range are particles of molecular size which, when dispersed in a solvent, may exist in true solution; at the other end of the range are particles which do not dissolve but form a suspension when dispersed in a liquid medium.) The objective of the centrifugation may be to obtain a clear supernate or a higher concentration of the particles. The present generation of high speed centrifuges routinely produce force fields of the order of 100,000 to 500,000 G's; however, even in the presence of such relatively strong centrifugal force fields, particles whose sedimentation coefficients are less than 10² Svedberg units require a period of hours or even days to concentrate or remove completely. Obviously, any reductions which may be effected in the periods required for centrifugation are considered very desirable.

Accordingly, it is an overall object of the present invention to provide apparatus and articles for reducing the time of centrifugation by increasing the rate of particle sedimentation or flotation.

Generally, it has been observed that in angle-head rotors, that is, rotors having sample receptacles, such as test tubes, inclined to the axis of rotation, precipitate is initially collected more rapidly than if the "wall effects" provided by the angle-head rotor were not present. It is known that in the case of particle suspensions, part of this action is the result of the accumulation of the particles against the inclined solid surfaces in the sample receptacles of the angle-head rotor. An aggregation of such particles then behaves as though it were a single, larger particle and sediments rapidly, sliding along the inclined surface to the most centrifugal (or in the case

of particle flotation, to the most centripetal) point of the receptacle holding the suspension. With respect to macromolecular particulate matter (for example, protein) which may be considered capable of existing in true solution in a solvent, the mechanism is thought to be somewhat different. An accepted explanation is that sedimentation of macromolecules causes the solution to become more concentrated adjacent the centrifugal wall of the sample receptacle and hence more dense. Bulk solution then flows downwardly along the inclined wall in the direction of higher centrifugal force thereby conveying the solute, that is, the macromolecular matter, along with it. The solution adjacent the centripetal wall of the receptacle, being somewhat depleted of solute and therefore relatively less dense, flows upwardly along the centripetal wall. A convection current is thereby created which, at least initially, speeds the precipitation of the macromolecular matter.

It is another object of the present invention to increase the rate at which particles can be removed from a sample solution or suspension carried in an angle-head rotor.

In general, the objects of the invention are accomplished by subdividing a portion of the sample receptacle volume into a number of small volumes, each provided with its own solid surface inclined to the direction of the centrifugal force field to receive centrifuged particulate material.

In accordance with one specific, exemplary form of the invention shown and described herein, the sample receptacles of a centrifuge rotor, which may simply be the recesses formed in the rotor or test tubes carried by the recesses, are each divided into a particle collection zone and a zone containing a plurality of compartments or cells in communication with the collection zone. The division of a portion of the receptacle into cells is obtained by providing at least one partition having a solid surface at least a portion of which is inclined to the direction of the centrifugal force. The partition may be made integral with the receptacle, or preferably, may be constructed in the form of a removable insert. Cross-sectionally, the individual cells may take any convenient shape—for example, rectangular, hexagonal or circular. The insert may be made as an integral unit or may be built up by joining, in any appropriate manner, individual elements such as small plastic tubes. They may be fabricated of materials having densities substantially that of the sample solution or suspension in which they are to be used. With a density slightly less than the sample the insert will tend to float thereby permitting collection, in the portion of the receptacle below the insert, of particles which are denser than the medium in which they are dispersed. Where it is desired to collect, by flotation, particles which are less dense than the particle-containing medium, the insert may be fabricated of a material of a slightly greater density than the sample, the particles then collecting in a portion of the receptacle above the insert.

The novel features which are believed to be characteristic of the invention are set forth with particularity in the appended claims. The invention itself, however, together with further objects and advantages thereof, can best be understood by reference to the following description taken in connection with the accompanying drawings in which:

FIG. 1 is an elevation view, in section, of an angle-head centrifuge rotor with a test tube and insert in accordance with one exemplary embodiment of the present invention;

FIG. 2A is a view, in section, along the plane 2—2 in FIG. 1;

FIG. 2B is a view, in section, along the plane 2—2 in FIG. 1 showing an alternative, exemplary embodiment of the present invention;

FIG. 3 is a perspective view of a test tube and insert in accordance with the embodiment depicted in FIGS. 1 and 2A; and

FIG. 4 is a perspective view of a test tube and insert in accordance with the alternative embodiment depicted in FIG. 2B.

Referring now to FIG. 1, there is shown an angle-head centrifuge rotor 10 mounted for rotation about its vertical axis 12. A suitable drive means (not shown), comprising generally an electric motor and transmission, provides the motive power to a drive shaft 14 supporting the rotor 10.

The rotor 10 is provided with one or more recesses 20 inclined with respect to the rotor axis 12. Each recess 20 has a "centrifugal" wall, that is, the wall remote from the axis of rotation and a "centripetal" wall which is the wall proximate the axis of rotation. Either or both walls may be inclined with respect to the axis of rotation. In the exemplary embodiment illustrated in FIG. 1, the most commonly employed configuration is depicted. The centrifugal and centripetal walls are both inclined to the rotational axis 12 and parallel to each other, the lower end of the recess 20 being further from the axis of rotation than the upper end. The point of greatest centrifugal force in the recess, at which precipitate heavier than the suspended medium or solvent will accumulate, is designated by the reference numeral 22.

The recess 20 is adapted to accommodate a removable test tube 24 made of metal, plastic or other material, in which the sample to be centrifuged is carried and in which the precipitated particles are collected. As already indicated, the sample may be carried directly in the recess 20 of the rotor; however, the use of a receptacle such as the test tube 24 not only serves to prevent interaction between the solution and the walls of the recess but makes more convenient the loading and removal of the sample material.

To prevent spillage and leakage of the contents of the test tube 24 during centrifugation, the mouth of the test tube is provided with a sealing cap designated generally by the reference numeral 26. The cap 26 may typically comprise a stem 28, a crown 30 and a flat resilient washer 32 sandwiched between the stem and the crown. The stem 28 may be provided with a depending flange 40, the outer surface of which is dimensioned for a close fit with the upper, interior wall surface of the test tube 24. The purpose of this flange is to provide support for the upper part of the test tube to prevent buckling or deformation under high "G" loadings. A stud 44, projecting upwardly from the stem 28 through a hole in the crown, is provided along with a nut 46 for tightening the cap 26 over the mouth of the test tube.

In accordance with the present invention, the interior of the test tube (or the interior of the recess 20 itself if no test tube is employed), is divided generally into a particle collection zone 50 and an adjacent zone 52 containing a plurality of compartments or cells 54 in communication with the collection zone 50. In the specific, exemplary embodiment depicted in FIGS. 1, 2A and 3, the particle collection zone 50 comprises the lower portion of the test tube 24 and the compartmented zone 52 comprises generally the upper portion of the test tube. This configuration is utilized where the particles are of greater density than the medium in which they are dispersed and thereby tend to move in the direction of increasing centrifugal force.

In the embodiment of FIGS. 1, 2A and 3, the cells 54 are defined by a number of partitions 56 arranged to form a grid-like pattern (best shown in FIGS. 2A and 3), each of the cells 54 being generally rectangular in cross-section and extending lengthwise substantially parallel to the side walls of the test tube 24. It will be appreciated that according to the invention, any number of cells may be provided and in the simplest form of the invention, only one partition, having a surface at least a portion of

which is disposed obliquely with respect to the direction of the centrifugal field in the recess, need be provided. In the simplest embodiment just described, some of the particles will accumulate or concentrate adjacent the centrifugal, or outermost, interior surface of the test tube and slide or flow toward the particle collection zone along that surface. The remaining part of the particles will accumulate or concentrate next to the portion of the partition surface which is oblique to the direction of the centrifugal field and move along that surface toward the zone 50. It is to be noted however, that the rates of particle separation and removal appear to increase as the number of cells in a test tube of given diameter is increased. The cross-sectional shape of the compartments is essentially immaterial and the invention will provide satisfactory results whether rectangular, hexagonal, circular, or any other shape is employed although, in particular applications, certain shapes may prove to be somewhat more efficient than others. It is therefore to be understood that the scope of this invention is not limited to any specific cell cross-section. One advantage of using rectangular cells is that the volume of solid material comprising the partitions can be minimized so that the sample volume is not reduced as much as when the cells are circular in cross-section. In one typical case, for example, using the same size test tube, there was approximately a 16% reduction in the liquid volume with a rectangular hole insert and about a 50% reduction using an insert with circular holes.

The partitions 56 may be made integral with the sample solution receptacles; however, there are certain advantages in making the partitions in the form of a removable insert such as the grid-like insert 58 shown in FIGS. 1, 2A and 3. One important advantage is that by the appropriate choice of material, the insert may be made to float or sink in the sample so that particles may be collected by either sedimentation or flotation.

With respect to insert materials, any suitable substance of adequate structural strength and resistance to interaction with the sample may be utilized. Inserts made of certain polyolefins have been successfully used with aqueous solutions since these materials have densities close to water. An example of a polyolefin used in solution in which the solvent was water is high-density polyethylene. The density of this substance is about 0.96 gm./cm.³ and it has considerable structural rigidity. The small density difference between the insert material and water results in only a small buoyant force on the insert. This, in combination with the inherent stiffness of the material, results in a structure which is self-supporting even when thin-walled and resists crushing, distortion and flow in high centrifugal force fields, for example, of the order of 230,000 G's. Low-density polyethylene may also be used, although this material has a tendency to "slow flow" in very high "G" fields.

A material of greater density than water and which can withstand extended periods of centrifugation without deforming, is polyethylene containing titanium dioxide pigment. The density of this material is about 0.5% greater than water. Another material which is denser than water and which exhibits adequate strength is phenolic-impregnated glass cloth. This material, although considerably more dense than water, will not deform in centrifugal force fields produced at rotational speeds as high as 65,000 r.p.m. From the standpoint of chemical inertness however, polyolefins, such as, for example, polyethylene, polypropylene and polybutylene appear to be best. The inserts may be fabricated in any manner, as for example, by extrusion, injection molding, or vacuum-forming.

FIGS. 2B and 4 show an exemplary embodiment of the present invention in which an insert 60 is made in the form of a cylinder provided with a series of longitudinal, spaced, parallel through-passages 62. This type of insert may be made by molding, extrusion or by fusing together

numerous low-density polyethylene tubes. In a typical cylindrically-shaped insert for utilization with a $\frac{5}{8}$ -inch diameter test tube, there is sufficient room for about 19 $\frac{3}{32}$ -inch holes or 38 $\frac{1}{16}$ -inch holes.

To prevent interference between the upper portion of the insert and the depending flange 40 of the stem 28, a portion of the length of the rectangular insert, designated by the reference numeral 64, may be cut back as shown in FIGS. 1 and 3. With respect to the cylindrical insert 60, the stem flange 40 can be eliminated since the insert itself provides adequate support for the test tube. In addition, a small amount of clearance should be provided between the insert and the receptacle wall to permit the insert to be introduced and removed with little effort. However, the clearance should be minimized so as not to create additional sedimentation spaces between the insert and the test tube wall.

With the aid of FIG. 1 of the drawings, the operation of the present invention will now be described. The receptacle, such as the test tube 24, is completely filled with a sample from which it is desired to separate or collect the suspended or dissolved particles. Assuming that the particles to be collected are denser than the medium in which they are suspended or dissolved, an insert with a density slightly less than the sample, that is, one which would not tend to centrifuge to the bottom of the tube, is utilized. A rectangular insert then will assume the position generally shown in FIG. 1 in which the lower edge of the cut back portion 64 of the insert abuts the lower edge of the depending flange 42. In the case of the cylindrical insert 60, it will move all the way up and come in contact with the lower surface of the stem 28.

The path of a typical suspended particle P is shown by the solid line. The particle sediments outwardly through the bulk solution along a radius of the rotor until it contacts one of the partitions 56. The particle continues to sediment along the surface of the partition 56 and along its way accumulates with other particles to form an aggregation of particles which behave like a single, large particle. The particle aggregation, upon reaching the lower edge of the partition, slides off the partition and continues outwardly along a radial path to form, along with other particle aggregations, a pellet 74 in the outermost point of the particle collection zone 50. Without the presence of the insert, the particle P, starting at the same point, would sediment along the path indicated by the dotted line until it reached the centrifugal wall of the test tube 24. Aggregations of particles, including the particle P, would then sediment along the test tube wall until the outermost point of the test tube was reached. Although the geometry would indicate that the distance traveled by the particle P in either case is the same, nevertheless, the particle P has traveled a much shorter distance as a single particle with the insert in place. The preceding description holds equally true for suspended particles collected by flotation.

If the material in the test tube is a true solution in which it is desired to isolate or remove from a solvent such as water a solute comprising macromolecules, such as protein, the presumed mechanism is that explained earlier. The presence of the insert hastens the circulation or convection. In this connection, the insert provides more solid surfaces which permit convection to take place throughout the solution rather than merely against the receptacle wall.

What is claimed is:

1. A receptacle for holding a sample including a medium with particles dispersed therein and effecting rapid separation and removal of said particles, said receptacle adapted to be carried by an angle-head centrifuge rotor mounted for rotation at high angular velocities at an angle with respect to the axis of rotation, comprising

a test tube having a side wall, at least a portion of which comprises a centrifugal wall, said test tube further having a particle collection zone and an adjacent zone containing a plurality of compartments in communication with said collection zone, said compartments being defined by at least one partition mounted in said test tube, said partition being positioned substantially parallel to said centrifugal wall of said tube and at an oblique angle with respect to the direction of centrifugal force caused by the rotation of said rotor.

2. A receptacle as defined in claim 1 in which said partition defining said plurality of compartments is in the form of a removable insert.

3. A receptacle as defined in claim 2 in which said insert comprises an integral structure of one group of spaced, planar partitions disposed substantially at right angles to another group of spaced, planar partitions thereby forming numerous elongated compartments substantially rectangular in cross-section.

4. A receptacle as defined in claim 2 in which said insert is substantially cylindrically-shaped having its longitudinal axis substantially parallel to said side wall of said test tube and includes a group of through-passages of circular cross-section extending substantially parallel to said axis.

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