

(19) **DANMARK**

(10) **DK/EP 3464550 T3**



(12) **Oversættelse af
europæisk patentskrift**

Patent- og
Varemærkestyrelsen

-
- (51) Int.Cl.: **C 12 M 1/24 (2006.01)** **C 12 M 1/06 (2006.01)**
- (45) Oversættelsen bekendtgjort den: **2020-04-06**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2020-03-11**
- (86) Europæisk ansøgning nr.: **17729310.7**
- (86) Europæisk indleveringsdag: **2017-05-31**
- (87) Den europæiske ansøgnings publiceringsdag: **2019-04-10**
- (86) International ansøgning nr.: **US2017035092**
- (87) Internationalt publikationsnr.: **WO2017210236**
- (30) Prioritet: **2016-05-31 US 201662343321 P**
- (84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**
- (73) Patenthaver: **Corning Incorporated, 1 Riverfront Plaza, Corning, New York 14831, USA**
- (72) Opfinder: **SCIBEK, Jeffery Joseph, 15 Highview Circle, Horseheads, New York 14845, USA**
WALCK, James Clark, Jr., 1065 Queen Esther Drive, Sayre, Pennsylvania 18840, USA
- (74) Fuldmægtig i Danmark: **NORDIC PATENT SERVICE A/S, Bredgade 30, 1260 København K, Danmark**
- (54) Benævnelse: **BEHOLDERE OG SPINNERKOBLER MED REDUCERET HJULSLØR TIL DYRKNING AF CELLER**
- (56) Fremdragne publikationer:
WO-A1-2009/009771
US-A- 3 649 465
US-A1- 2008 131 957

DESCRIPTION

TECHNICAL FIELD

[0001] The present disclosure relates generally to spinner flasks and vessels for culturing cells. More particularly, embodiments described herein relate to disposable spinner flask vessels having an impeller assembly.

BACKGROUND

[0002] In various scientific fields, it is useful to grow cells in a culture media (such as a liquid suspension) over an extended period of time. However, increasing cell numbers result in the depletion of nutrients in the culture medium. When the media is allowed to stagnate, cell growth is inhibited due to the local depletion of nutrients and the cells can die.

[0003] In general, it is understood that a cell culture suspension must be stirred in order to effectively grow cells. Optionally, microcarriers to which cells may be attached may be suspended in cell culture media. A spinner flask is a type of cell culture vessel that employs a suspended impeller driven by an external rotating magnet under the base of the spinner flask to maintain the cells in suspension. The design of the suspended impeller is complicated since many different factors must be balanced to properly influence the suspension of cell cultures such as the hydrodynamic stress imparted on growing cells that can damage them or alter their morphology. In addition, the blade design and movement must keep the cells suspended but not shear the cells or the microcarriers.

[0004] Further, care must be taken not to contaminate the culture media. Spinner flasks have typically consisted of glass and metal reusable cell culture vessels comprising an amalgam of working parts each of which requires cleaning, sterilization (usually by autoclave) and proper storage between uses. When such an amalgam of working parts is used for a spinner flask, the user can spend considerable time keeping track of the parts and maintaining the proper conditions for culturing cells.

[0005] WO2009009771 A1 discloses a spinner flask with a plurality of planar rotor blades and a magnetic device, wherein one central protrusion at the bottom of the vessel allows the central impeller to rotate but prevents lateral movement of the impeller when it is rotating. US2008131957 A1 refers to a cell culture stirring vessel and impeller comprising an integral vessel body having a top surface, bottom surface and a substantially cylindrical sidewall, an impeller assembly having a plurality of planar blades, a central axis, a flexible shaft extending from the top surface, a magnet receptacle formed within the plurality of blades and a magnet nested within the magnet receptacle, wherein the plurality of blades extend orthogonally from the central axis and wherein the shaft rotates around the central axis.

[0006] There accordingly remains a need for an inexpensive, disposable, pre-sterilized, fully integrated cell culture vessel which provides gentle stirring to keep cells suspended and prevent shearing.

SUMMARY

[0007] Described herein are various embodiments of a disposable spinner flask vessel for culturing cells having an impeller assembly stabilized by a plurality of positioning nubs.

[0008] According to an aspect of the present disclosure, a vessel for culturing cells is provided that includes a vessel body having a top portion, a bottom portion having a bottom interior surface, and a cylindrical sidewall. The vessel additionally has an impeller assembly inside the vessel body having a top portion rotatably coupled to the top portion of the vessel body, the impeller assembly having a plurality of planar blades, a central axis, a flexible shaft extending down from the top portion of the impeller assembly, a magnet receptacle molded within the plurality of planar blades, a magnet within the magnet receptacle, and an impeller o-ring coupled to a bottom surface of the planar blades. The vessel also includes a plurality of positioning nubs coupled to the bottom interior surface of the vessel body, spaced from an inside edge of the impeller o-ring.

[0009] According to another aspect of the present disclosure, a vessel for culturing cells is provided that includes a vessel body having a top portion, a bottom portion having a bottom interior surface, and a cylindrical sidewall. The vessel also includes an impeller assembly inside the vessel body having a top portion rotatably coupled to the top portion of the vessel body, the impeller assembly having a plurality of planar blades, a central axis, a flexible shaft extending down from the top portion of the impeller assembly, a magnet receptacle molded within the plurality of planar blades, a magnet within the magnet receptacle, and an impeller o-ring coupled to a bottom surface of the planar blades. The vessel further includes a plurality of positioning nubs coupled to the bottom interior surface of the vessel body, spaced from an inside edge of the impeller o-ring; and a center nub coupled to the bottom interior surface of the vessel body coincident with the central axis of the impeller assembly.

[0010] Additional features and advantages will be set forth in the detailed description which follows, and in part will be readily apparent to those skilled in the art from that description or recognized by practicing the embodiments as described herein, including the detailed description which follows, the claims, as well as the appended drawings.

[0011] The accompanying drawings are included to provide a further understanding, and are incorporated in and constitute a part of this specification. The drawings illustrate one or more embodiments, and together with the description serve to explain principles and operation of the various embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012]

FIG. 1 is a cut-away perspective view of an embodiment of a spinner flask;

FIG. 2 is an exploded view of the flask depicted in FIG. 1;

FIG. 3 is a cut-away top view of a spinner flask showing a lower impeller assembly and a plurality of positioning nubs according to an embodiment;

FIG. 4 is a cut-away side view of a lower impeller assembly and a plurality of cylindrical positioning nubs according to an embodiment;

FIG. 5 is a cut-away side view of the bottom of an impeller assembly and a plurality of conical positioning nubs according to an embodiment;

FIG. 6 is an enlarged cut-away side view of the impeller assembly and positioning nubs depicted in FIG. 5 according to an embodiment;

FIG. 7 is a top perspective view of an embodiment of a spinner flask;

FIG. 8 is a cross section view taken along the A-A' axis of FIG. 4;

FIG. 9 is a cross section view taken along the A-A' axis of FIG. 4 rotated 90°;

FIG. 10 is an enlarged view of the upper impeller assembly and shaft;

FIG. 11 is a bottom perspective view of an embodiment of a spinner flask;

FIG. 12A is an image showing Cytodex-1 microcarriers cultured in a non-disposable 1L glass spinner flask;

FIG. 12B is an image showing Cytodex-1 microcarriers cultured in a commercially available 1L disposable spinner flask;

FIG. 13A is an image showing enhanced attachment microcarriers with Vero cells cultured in a non-disposable 1L glass spinner flask;

FIG. 13B is an image showing enhanced attachment microcarriers with Vero cells cultured in a commercially available 1L disposable spinner flask according to an embodiment;

FIG. 14A is a prophetic image showing Cytodex-1 microcarriers cultured in an embodiment of a 1L disposable spinner flask with a plurality of positioning nubs; and

FIG. 14B is a prophetic image showing enhanced attachment microcarriers with Vero cells cultured in an embodiment of a 1L disposable spinner flask with a plurality of positioning nubs.

DETAILED DESCRIPTION

[0013] As used herein, the term "and/or," when used in a list of two or more items, means that any one of the listed items can be employed by itself, or any combination of two or more of the listed items, can be employed. For example, if a composition is described as containing components A, B, and/or C, the composition can contain A alone; B alone; C alone; A and B in combination; A and C in combination; B and C in combination; or A, B, and C in combination.

[0014] In this document, relational terms, such as first and second, top and bottom, and the like, are used solely to distinguish one entity or action from another entity or action, without necessarily requiring or implying any actual such relationship or order between such entities or actions. The terms "comprises," "comprising," or any other variation thereof, are intended to cover a non-exclusive inclusion, such that a process, method, article, or apparatus that comprises a list of elements does not include only those elements but may include other elements not expressly listed or inherent to such process, method, article, or apparatus. An element preceded by "comprises ... a" does not, without more constraints, preclude the existence of additional identical elements in the process, method, article, or apparatus that comprises the element.

[0015] Several currently available large-volume spinner flasks have been demonstrated to be incompatible with microcarrier cultures. Use of these flasks result in physical damage to both microcarriers and attached cells. A root cause for this damage to cells, cells attached to the microcarriers, and/or the microcarrier's themselves is believed to be due to impeller contact between a central nub and other features of the vessel. Insufficient clearance between a bottom surface of the impeller blade and the top of a raised feature on the bottom of the vessel can also cause cell damage.

[0016] Referring to FIGS. 1-11, a vessel 4 for culturing cells is shown in exemplary form according to aspects of the disclosure. The vessel 4 comprises a vessel body 8 having a top portion 12 and a bottom portion 16, one or more necked access ports 32, and an impeller assembly 58. The top portion 12 and the bottom portion 16 are circumferentially sealed along a weld line 20 which is the result of a joining of a top interconnecting lip 34 and a bottom interconnecting lip 38 circumscribing the periphery of both portions. The vessel 4 has a substantially cylindrical shape and a central axis A-A' with an inner surface, an outer surface, a top part 74, a sidewall 10, and a bottom interior surface 70 having a center nub 112. In certain aspects the center nub 112 can be a cylindrical center nub 114 (FIG. 4) or a conical center nub 118 (FIG. 5). Although any size vessel is theoretically possible, the sizes for stirring vessels of aspects of the disclosure typically range from 125 ml to 10 liters and specific sizes include a one liter, two liter, and a three liter version.

[0017] The impeller assembly 58 includes a center shaft 46 extending along a A-A' central

axis. In different aspects of the present disclosure, the center shaft 46 may be flexible, rigid, or possess other varying amounts of flexibility. Extending from and contiguous with the shaft 46 are four planar blades 50, 54 each disposed 90 degrees relative to each other. Of the four planar blades 50, 54 there are two major blades 50 and two minor blades 54. The major blades 50 are disposed 180 degrees relative to one another and likewise, the two minor blades 54 are disposed 180 degrees relative to each another. The arrangement of blades 50, 54 around the central shaft 46 creates an alternating effect of a minor-major blade orientation. It is believed that this orientation provides enhanced mixing of fluid in both the lateral as well as vertical planes within the vessel 4. The blades 50, 54 represent planes that are oriented vertically when the vessel 4 is sitting upright. As understood by those with ordinary skill in the art, other blade configurations, shapes, and arrangements can be employed in this disclosure, including those that employ fewer or more than four blades.

[0018] With reference to FIGS. 3-6, at the bottom of the shaft 46 and beneath the major and minor blades 50, 54 of the impeller assembly is an impeller o-ring 102. The impeller o-ring 102 is coupled to a bottom cut-out portion 86 of the planar blades and the bottom edge of the impeller o-ring may be even with a bottom edge 66 of the planar blades 50, 54. A plurality of positioning nubs 98 are coupled to the bottom interior surface 70 spaced from or along an inside edge 102a of the impeller o-ring 102. The group of positioning nubs can have two, three, four, five, six, or more positioning nubs 98 spaced apart from each other along the inside edge 102a of the impeller o-ring 102. The plurality of positioning nubs 98 may be spaced an equal distance from one another along the inside edge 102a of the impeller o-ring 102. In an embodiment, the plurality of positioning nubs is three positioning nubs 98, each nub spaced an equal distance from one another. The plurality of positioning nubs can have each nub spaced an equal distance from one another along the inside edge 102a of the impeller o-ring 102.

[0019] The plurality of positioning nubs 98 may comprise individual nubs that have a cylindrical, block, square, conical, rectangular, or pyramidal shape. The plurality of positioning nubs 98 can also have other shapes or combinations of shapes. In FIG. 4, for example, the plurality of positioning nubs 98 is shown as cylindrical positioning nubs 106. In FIGS. 5-6, the plurality of positioning nubs 98 is shown as conical positioning nubs 110. In certain aspects, the shape of the positioning nubs 98 can be configured to make constant, periodic, or frequent contact with the inside edge 102a of the impeller o-ring 102 to prevent perpendicular or lateral movement of the impeller assembly 58. The plurality of positioning nubs 98 minimize impeller wobble thus reducing contact between the impeller assembly 58 and the center nub 112 positioned at the bottom interior surface 70 of the vessel 4.

[0020] The center nub 112 is a raised feature as shown in FIGS. 4-6 and can comprise various shapes. For example, the center nub 112 can be configured as a cylindrical center nub 114, as shown in FIGS. 4 and 6. In another embodiment, the center nub 112 can be configured as a conical center nub 118, as shown in FIG. 5. The center nub 112 may include cylindrical, block, square, conical, rectangular, pyramidal, or any other shaped nub. In some embodiments, the center nub 112 can have rounded or beveled edges. The center nub 112 serves at least two

purposes: 1) to prevent microcarriers and/or cells from pooling or grouping together below the impeller assembly 58; and 2) to minimize the perpendicular or lateral movement of the impeller assembly 58 during shipment and spinning to avoid impeller breakage. Although the raised center nub 112 can help minimize the perpendicular or lateral movement of the impeller assembly 58, the positioning nubs 98 may also help minimize the perpendicular or lateral movement of the impeller assembly 58.

[0021] In some aspects, the distance between the top surface of the center nub 112 and the bottom of the impeller assembly 58 can be from about 0.001 inches to about 0.1 inches. In other aspects, the distance between the top of the center nub 112 and the bottom of the impeller assembly 58 can be from about 0.005 inches to about 0.05 inches. In still other aspects, the distance between the top of the center nub 112 and the bottom of the impeller assembly 58 can be from about 0.01 inches to about 0.03 inches. For example, the distance between the top of the center nub 112 and the bottom of the impeller assembly 58 can be about 0.0150 inches, about 0.0185 inches, about 0.0200 inches, about 0.0250 inches, or all distance values between these recited values and ranges disclosed herein. The tolerance for the plurality of positioning nubs 98, the center nub 112, the impeller assembly 58, and other dimensional features of vessel 4 is about +/- 0.005 inches. In some aspects, the tolerance for the plurality of positioning nubs 98, the center nub 112, the impeller assembly 58, and other dimensional features of vessel 4 is from +/- 0.001 inches to +/- 0.100 inches.

[0022] In some aspects, the distance between the base outer diameter (diameter of the center nub where connected to bottom interior surface) of the center nub 112 and the inner diameter of the impeller o-ring 102 can be from about 0.01 inches to about 1.0 inches. In other aspects, the distance between the base outer diameter of the center nub 112 and the inner diameter of the impeller o-ring 102 can be from about 0.1 inches to about 0.5 inches. In still other aspects, the distance between the base outer diameter of the center nub 112 and the inner diameter of the impeller o-ring 102 can be from about 0.2 inches to about 0.3 inches. For example, the distance between the base outer diameter of the center nub 112 and the inner diameter of the impeller o-ring 102 can be about 0.15 inches, about 0.20 inches, about 0.025 inches, about 0.030 inches, about 0.035 inches, or all distance values between these recited values and ranges disclosed herein.

[0023] The spinner flasks and vessels for culturing cells disclosed herein have the ability to offer a readily available, affordable, disposable, pre-sterilized, fully integrated cell culture vessel 4 which provides gentle stirring to minimize hydrodynamic shearing and keep cells suspended in the vessel 4. A center nub 112 and/or positioning nubs 98 on the bottom interior surface 70 of the flask can prevent pooling of microcarriers and/or cells below the impeller assembly 58 and also can minimize the perpendicular movement of the impeller assembly 58 during shipping and spinning to prevent damage to the microcarriers, cells, and/or impeller assembly 58.

[0024] With reference to FIG. 10, the center shaft 46 has a substantially tapered top end 126 that is mounted in the top portion 12 of the vessel 4 by contact with a shaft receptacle 138

centrally located and integral with the top part 74 of the top portion 12 of the vessel 4. An o-ring 82 is located upon a receiving groove 130 on the shaft 46. The o-ring 82 may be made from PTFE, nylon or other similar low-friction material. The o-ring 82 rests against a circular shelf 134 in such a way that the impeller assembly 58 hangs freely within the vessel 4. By having this single mounting point in the shaft receptacle 138, the flexible shaft 46 is free to rotate. A disc top 122 in the top part 74 seals the shaft receptacle 138 from the external environment. The center shaft 46 further advantageously has a plurality of horizontal support ribs 142 that enhance the stiffness of the shaft 46. The shaft receptacle 138 has sidewalls that extend into the vessel 4 creating a sleeve 146 for receiving the shaft. The sleeve 146 maintains the shaft's 46 orientation within the central axis A-A' and prevents any lateral movement of the impeller assembly 58 within the vessel 4.

[0025] The bottom cut-out portion 86 of the impeller assembly 58 is shaped within the intersection of the bottom edges 66 of the respective blades 50, 54. The bottom cut-out portion 86 substantially follows the contour of the cylindrical center nub 114 or conical center nub 118. The impeller o-ring 102 coupled to the bottom cut-out portion 86 of the impeller assembly 58 is not intended to contact the cylindrical and conical center nubs 114, 118 when the vessel 4 is positioned in an upright position. In fact, there may be no contact points between the impeller assembly 58 and the vessel 4 below the shaft receptacle 138. This is advantageous in that it helps reduce the possibility of cell damage if cells, microcarriers, or cells cultured on microcarriers are caught between the bottom edges 66 of the blades 50, 54 and the center nubs 114, 118, or due to shear. The cylindrical and conical center nubs 114, 118 on the bottom interior surface 70 also eliminate any potential dead spots (spots where turbulence created by the spinning impeller is at a minimum) directly below the central axis A-A' of the impeller assembly 58. The bottom cut-out portion 86 within the intersection point of the blades 50, 54 allows the blade edges 66 to come into close proximity with the bottom interior surface 70. In an aspect, the distance between the blades 50, 54 and the bottom interior surface 70 is between about 0.05 inches and about 0.5 inches. Since the vessel 4 is intended to be shipped as an integral unit, the impeller o-ring 102 in combination with the center nub 112 also serve to contain the impeller 58 during shipping in such a way that the impeller assembly 58 cannot disengage from the bottom of the vessel 4 or damage the sidewall 10 through contact caused by jostling of the vessel 4.

[0026] Referring specifically to FIG. 8, which is a vertical cross section of the vessel 4 of an embodiment, each major blade 50 includes an upper portion 52 and a lower portion 60. The upper portion 52 has a substantially triangular shape with an outer edge progressively angled away from the shaft 46 and central axis A-A'. The lower portion 60 of each major blade 50 is substantially rectangular in shape. Referring specifically to FIG. 9, which is a cross section of the vessel 4 taken 90 degrees from the section represented by FIG. 8, the minor blades 54 form a triangular shape along their entire length. An outer edge 48 of each minor blade 54 extends linearly away from the central axis A-A' to a point at the bottom edge 66 of the blades 50, 54. The bottom edges 66 of the lower portion of all blades 50, 54 (minor and major) extend generally parallel to the bottom interior surface 70 of the vessel, but do not contact the bottom interior surface 70. The impeller assembly 58 including blades 50, 54 may be made from

polypropylene, TPE, a silicone, or other appropriate polymeric materials.

[0027] A magnet receptacle 62 for receiving a magnetic stir bar (magnet) 94 is molded into each lower portion 60 of the two major blades 50. A hole 56 in the minor blades 54 and shaft 46 area completes the magnet receptacle 62. A cylindrical plug or magnetic stir bar 94 is mounted in the magnet receptacle 62 along the lower edge of the two major blades 50 and orthogonal to the minor blades 54. Alternatively, the magnet 94 itself is molded into the impeller assembly 58. To accomplish this, the magnet 94 is inserted into a mold and the impeller assembly 58 is over-molded around the magnet 94 itself. Having the magnet 94 integrally molded within the impeller assembly 58 provides the advantage that during assembly and shipping, the magnet 94 cannot detach from the impeller assembly 58 and damage the vessel body 8.

[0028] The one or more access ports 32 extend outward from the top portion 12 of the vessel 4. Optional internally threaded sealing caps 28 are installed on exteriorly threaded access ports 32. In an embodiment, the sealing cap 28 has within it a hydrophobic membrane insert 90 made from material that will allow gas transport into the vessel interior but prevent liquid from escaping the vessel 4 and other contaminants from entering the vessel 4. Examples of such membrane material include polytetrafluoroethylene and polyvinylidene fluoride (PVDF). In some aspects, the sealing caps 28 further have a vent 30 that allows the necessary gaseous communication with the external environment. In another aspect, accessories such as tubes may be employed and connected to the one or more access ports 32 to allow aseptic dispensing.

[0029] In an aspect, the one or more access ports 32 extend at an angle from horizontal to allow instruments such as pipettes to pass by impeller assembly 58 and reach adjacent regions of stirring vessels having preselected depths. Nevertheless, the dimensions of the one or more access ports 32 and the angles in which the access ports 32 extend from the vessel body 8 may be selected to optimize instrument accessibility to regions within various vessels 4. Further, in some aspects, the one or more access ports is two access ports 32 as disclosed in the FIGS., but it will be appreciated that any number of ports 32 are possible.

[0030] A plurality of baffles 24 extend along the interior wall in a vertical direction which is parallel to the central axis A-A'. Each baffle 24 has roughly the cross sectional shape of a half-cylinder or an isosceles triangle. Each baffle 24 originates from the vessel bottom 70 and extends vertically upward terminating in an elliptical shape 26. While the baffles 24 illustrated herein are shown to terminate in an elliptical shape 26, aspects of the present disclosure are not so limited, and any shape may be employed. It is believed that having the plurality of baffles 24 extend completely through the liquid region (i.e. from the bottom interior surface 70 to a point above the liquid surface) enhances turbulence throughout the entire liquid domain. The plurality of baffles 24 project into the vessel cavity, in combination with the impeller assembly 58, create and enable turbulence with the vessel interior. The plurality of baffles 24 are preferably formed integrally with the wall of the container. In some embodiments, the plurality of baffles 24 is three baffles 24, each baffle is spaced an equal distance from each of

the other baffles around a perimeter defined by the cylindrical sidewall. In other aspects, the plurality of baffles is disposed symmetrically along the interior cylindrical sidewall, but the number and density of baffles 24 may vary based on vessel size. In embodiments, each baffle is integral with the sidewall of the vessel body originating at the bottom interior surface and extending vertically to a predetermined distance up the sidewall.

[0031] In some embodiments, the vessel 4 of the present disclosure is made from an injection molded polymer, for example polystyrene, polycarbonate or any other appropriate polymer as identified by one of skill in the art. In an embodiment, the polymer is optically transparent and non-cytotoxic. Since the materials are made from lightweight polymers and the vessel 4 is pre-sterilized during manufacture, the vessel 4 itself is disposable and there is no need for the end user to sterilize components of the system prior to use.

[0032] In some aspects, the top portion 12 or the bottom portion 16 of the vessel sidewall 10, may have one or more areas that may contain gas permeable/liquid impermeable film or membrane. In aspects where this gas permeable/liquid impermeable film or membrane is located in an area of the vessel 4 that will contact the cell suspension, improved gas exchange with the external environment is achieved. As such, in some aspects, the areas are located in the lower half of the sidewall 10 or in the bottom interior surface 70.

[0033] In describing the manufacturing and assembly process, the impeller assembly 58, the top portion 12 and the bottom portion 16 of the vessel body 8 are molded separately and treated as discussed. Thereafter, the magnet 94 is placed in the magnet receptacle 62. As previously noted, in another aspect, the magnet 94 itself is over-molded and therefore integral with the impeller assembly 58. The impeller assembly 58 is placed within a shaft receptacle 42 of the top portion 12. The o-ring 82 is slipped over the top end of the shaft 46 and contacted with the receiving groove 130. The top portion 12 and the bottom portion 16 of the vessel 4 are then permanently affixed to one another by, for example, ultrasonic welding along the weld line 20 thereby creating a completely and permanently integral unit. Similarly, a disc top 122 is welded in position sealing the shaft receptacle 42. In other aspects, the parts are laser welded or attached by means of adhesives. In aspects having one or more necked access ports 32 and sealing caps 28, the sealing caps 28 are put into place and the unit is effectively sealed for shipment. The integral unit may then be sterilized. As most cell culture procedures are carried out under aseptic conditions by practicing the so-called sterile technique, the pre-sterilization of the vessel 4 provides the culture chamber to be maintained in a sterile, closed environment. It is advantageous to have the cell culture process carried out in a system where the culture chamber is functionally closed to the external environment, with the sterile integrity maintained from the time the device is manufactured until it has been disposed of. One method of pre-sterilizing includes gamma irradiation. Other sterilization methods known to those skilled in the art including ethylene oxide or electron beam irradiation treatment could also be used.

[0034] Based on the manufacturing approach and since the weld line 20 exists in the sidewall 10 region of the vessel 4, the impeller assembly 58 may be sized such that the blades 50, 54 extend nearly the full diameter of the vessel 4. In an aspect the impeller blades 50, 54 extend

approximately 50-95% of the vessel's radius, as measured from the central axis A-A' to the sidewall 10. In another aspect, at least one blade 50, 54 extends 75-95% of the vessel's radius but due to the design and manufacturing approach, may extend any distance.

[0035] To operate the system of the present disclosure, liquid (such as a culture media including cells) is delivered through the one or more access ports 32 of the container. The liquid is added until it reaches a fluid level which is preferably below the top edge of the blades 50, 54 and the top of the baffles 24 but above the lower portion 60 of the major blades 50.

[0036] Once the liquid is in the vessel 4, the vessel 4 is placed upon a magnetic stirring device (not shown) and the stirring device causes the magnetic stir bar 94 to spin within the vessel 4. As a result, the impeller assembly 58 including the shaft 46 and the blades 50, 54 is also caused to rotate within the vessel 4. The rotation of the assembly 58 causes the fluid to stir within the container. Alternatively, the impeller 58 may be rotated by a motorized mechanism engaging the top of the shaft 46. The shape of the blades 50, 54 and the interaction with the baffles 24 causes the liquid to circulate from a position near the top of the fluid level to a position near the bottom of the fluid level. The center nub 112 prevents material from accumulating at the center of the bottom interior surface 70. Since the upper portion 52 of the major blades 50 extends above the fluid level, the surface area of the liquid in the container is effectively increased and continually agitated, resulting in aeration of the liquid.

[0037] The apparatus is used to stir cells suspended in a culture medium. The cells may also be attached to microcarriers suspended in the culture medium. This mixing can be performed over a relatively long time (i.e., from several hours up to several months) but must not produce great stress to cells suspended in the liquid. The mixing must be effective such that the liquid cycles from the bottom of the apparatus to the surface, and back again. Typically, the cells are maintained at about 27 °C - 37 °C and mixed at 5 to 300 rpm. Of course, these conditions can be varied depending on the particular cells or application. Cells or cellular materials may be harvested through the access ports by means of pipette, pouring, or pumping.

According to an embodiment (1) of the present disclosure, a vessel for culturing cells is provided. The vessel comprises: a vessel body having a top portion, a bottom portion comprising a bottom interior surface, and a cylindrical sidewall; an impeller assembly inside the vessel body comprising a top portion rotatably coupled to the top portion of the vessel body, the impeller assembly having a plurality of planar blades, a central axis, a flexible shaft extending down from the top portion of the impeller assembly, a magnet receptacle molded within the plurality of planar blades, a magnet within the magnet receptacle, and an impeller o-ring coupled to a bottom surface of the planar blades; and a plurality of positioning nubs coupled to the bottom interior surface of the vessel body, spaced from an inside edge of the impeller o-ring.

According to an embodiment (2) of the present disclosure, the vessel of aspect (1) is provided, wherein the plurality of positioning nubs is three positioning nubs, each nub spaced circumferentially from each of the other nubs at an approximately equal distance.

According to an embodiment (3) of the present disclosure, the vessel of aspect (2) is provided, wherein each of the nubs have a conical shape.

According to an embodiment (4) of the present disclosure, the vessel of aspect (2) is provided, wherein each of the nubs have a cylindrical shape.

According to an embodiment (5) of the present disclosure, the vessel of any of aspects (1)-(4) is provided, wherein the vessel body comprises a polymeric material.

According to an embodiment (6) of the present disclosure, the vessel of any of aspects (1)-(5) is provided, wherein the plurality of planar blades is four planar blades coupled to the flexible shaft, each blade disposed at 90 degrees relative to the blades adjacent to it.

According to an embodiment (7) of the present disclosure, the vessel of any of aspects (1)-(6) is provided, further comprising: a plurality of baffles, each baffle integral with the sidewall of the vessel body originating at the bottom interior surface and extending vertically to a predetermined distance up the sidewall.

According to an embodiment (8) of the present disclosure, the vessel of aspect (7) is provided, wherein each baffle terminates in an elliptical shape.

According to an embodiment (9) of the present disclosure, the vessel of any of aspects (7)-(8) is provided, wherein the plurality of baffles is three baffles, each baffle is spaced an equal distance from each of the other baffles around a perimeter defined by the cylindrical sidewall.

According to an embodiment (10) of the present disclosure, the vessel of any of aspects (1)-(9) is provided, wherein the vessel body further comprises a radius measured by the distance from the central axis to the sidewall, and further wherein at least one of the blades extends about 50 to 95% of the radius of the vessel body toward the cylindrical sidewall.

According to an embodiment (11) of the present disclosure, the vessel of any of aspects (1)-(10) is provided, wherein each blade comprises a bottom edge spaced from about 0.05 inches to about 0.5 inches from the bottom interior surface.

According to an embodiment (12) of the present disclosure, the vessel of any of aspects (1)-(11) is provided, wherein the impeller assembly is sealed inside the vessel body.

According to aspect (13) of the present disclosure, a vessel for culturing cells. The vessel comprises: a vessel body having a top portion, a bottom portion comprising a bottom interior surface, and a cylindrical sidewall; an impeller assembly inside the vessel body comprising a top portion rotatably coupled to the top portion of the vessel body, the impeller assembly having a plurality of planar blades, a central axis, a flexible shaft extending down from the top portion of the impeller assembly, a magnet receptacle molded within the plurality of planar blades, a magnet within the magnet receptacle, and an impeller o-ring coupled to a bottom surface of the planar blades; a plurality of positioning nubs coupled to the bottom interior surface of the vessel body, spaced from an inside edge of the impeller o-ring; and a center nub coupled to the bottom interior surface of the vessel body coincident with the central axis of the

impeller assembly.

According to an aspect (14) of the present disclosure, the vessel of aspect (13) is provided, wherein the plurality of baffles is three baffles, each baffle is spaced an equal distance from each of the other baffles around a perimeter defined by the cylindrical sidewall.

According to an aspect (15) of the present disclosure, the vessel of any of aspects (13)-(14) is provided, wherein the vessel body further comprises a radius measured by the distance from the central axis to the sidewall, and further wherein at least one of the blades extends about 50 to 95% of the radius of the vessel body toward the cylindrical sidewall.

According to an aspect (16) of the present disclosure, the vessel of any of aspects (13)-(15) is provided, wherein the plurality of positioning nubs is three positioning nubs, each nub spaced circumferentially from each of the other nubs at an approximately equal distance.

According to an aspect (17) of the present disclosure, the vessel of aspect (16) is provided, wherein each of the nubs have a conical shape.

According to an aspect (18) of the present disclosure, the vessel of any of aspects (13)-(16) is provided, wherein the center nub has a cylindrical shape or a conical shape.

According to an aspect (19) of the present disclosure, the vessel of any of aspects (13)-(18) is provided, wherein the vessel body is a polymeric material.

According to an aspect (20) of the present disclosure, the vessel of any of aspects (13)-(19) is provided, wherein the plurality of planar blades is four planar blades coupled to the flexible shaft, each blade disposed at 90 degrees relative to the blades adjacent to it.

EXAMPLES

[0038] Embodiments of the present disclosure are further described below with respect to certain exemplary and specific embodiments thereof, which are illustrative only and are not intended to be limiting. The following Examples demonstrate the utility and effectiveness of using a plurality of positioning nubs 98 to minimize the perpendicular or lateral movement of the impeller assembly 58 to prevent causing damage to cells and microcarriers in a vessel for culturing cells.

Materials

[0039] The Cytodex-1 microcarriers (GE Healthcare # 17-0448-01) and enhanced attachment microcarriers (Corning # 3779) were used according to the manufacturer's recommendations. Vero cells (ATCC# CCL81™) were cultured in DMEM (Corning #10-010) supplemented with

2mL L-glutamine (Corning #25-005), IX MEM NEAA (Corning #25-025) and 10% fetal bovine serum (Corning #35-010). The commercially available 1L disposable spinner flasks (Corning #3580) and 1L glass spinner flasks (Corning #4500-1L) were supplied by Corning Incorporated.

Methods and Procedures

[0040] Microcarriers were added to spinner flasks at 10 cm²/mL in reduced-serum culture medium (\leq 0.5% serum). The flasks were equilibrated in a 5% CO₂ incubator at 37°C for 1 to 2 hours for media equilibration. Vero cells were seeded at a density of 15,000 cells/cm² and the culture was mixed continuously at 30 rpm during cell attachment. After the cells were attached to the microcarriers the media was adjusted to 5% serum. The culture was expanded for 5 days with a media exchange on day 3. Cells and microcarriers were visualized using a Zeiss Axiovert 40 C inverted light microscope at 5x and 10x magnification. Cells were quantified daily using a Nucleocounter NC-200 automated cell counter.

Comparative Example 1: Traditional Glass Spinner Flask Vessel and Disposable Spinner Flask Vessel with Microcarriers

[0041] The images shown in FIGS. 12A and 12B were both captured on Day 3 of the culture at 10x magnification. The FIG. 12A image shows Cytodex-1 microcarriers in a non-disposable 1L glass spinner flask and the FIG. 12B image shows the Cytodex-1 microcarriers in a commercially available 1L disposable spinner flask. FIG. 12A demonstrates that Cytodex-1 microcarriers can be successfully cultured in non-disposable 1L glass spinner flasks with no observable damage to the cells or microcarriers. The non-disposable 1L glass spinner flasks has a rigid impeller assembly that is secured to the top surface and is spaced from the bottom surface of the flask so that no contact is made between the impeller assembly and any additional surface of the flask. FIG. 12B demonstrates that the Cytodex-1 microcarriers are significantly damaged in the commercially available 1L disposable spinner flask. The images highlight that the commercially available 1L disposable spinner flask is incompatible with Cytodex 1 microcarriers.

Comparative Example 2: Traditional Glass Spinner Flask Vessel and Disposable Spinner Flask Vessel with Microcarriers having Cells

[0042] The images shown in FIGS. 13A and 13B were both captured on Day 3 of the culture at 10x magnification. The image in FIG. 13A displays several enhanced attachment microcarriers with a layer of Vero cells still layered on the surface in a non-disposable 1L glass spinner flask. FIG. 13A demonstrates that the non-disposable 1L glass spinner flask promotes cell growth to

the microcarriers since a healthy, confluent monolayer of cells can be observed. The image in FIG. 13B also displays several enhanced attachment microcarriers but here the layer of Vero cells are sheared from the surface from using the commercially available 1L disposable spinner flask. FIG. 13B demonstrates the damage caused to the Vero cells in the commercially available 1L disposable spinner flask since it can be observed in FIG 13B that the microcarriers have a majority of the cells removed from the surface as well as microcarriers with cells that are peeling off. The inherent wobble and contact of the impeller assembly of the commercially available 1L disposable spinner flask create the physical damage to the cells and microcarriers.

Prophetic Example 1: Disposable Spinner Flask with Positioning Nubs

[0043] This prophetic example modifies a commercially available 1L disposable spinner flask by adding a plurality of positioning nubs spaced from an inside edge of an impeller o-ring on an impeller assembly. As described in the embodiments of the Detailed Description above, utilizing a center nub positioned on the bottom of the vessel combined with a plurality of positioning nubs spaced from an inside edge of an impeller o-ring would prevent the perpendicular or lateral movement of the impeller thereby preventing the impeller wobble. Eliminating this unwanted impeller assembly movement will mimic the conditions of the non-disposable 1L glass spinner flask and minimize the damage done to microcarriers and microcarriers having cells shown in FIGS 12A and 13A. The prophetic image shown in FIG. 14A would be captured on Day 3 of the culture at 5x magnification and would be expected to display Cytodex 1 microcarriers in a 1L disposable spinner flask with a plurality of positioning nubs. The prophetic image shown in FIG. 14B would be captured on Day 3 of the culture at 10x magnification and would be expected to display Vero cells cultured on enhanced attachment microcarriers in a 1L disposable spinner flask with a plurality of positioning nubs. Both FIGS. 14A and 14B would be expected to show that Cytodex 1 microcarrier and Vero cell damage is visibly reduced or eliminated when impeller wobble is prevented by the center nub positioned on the bottom of the vessel and the plurality of positioning nubs spaced from an inside edge of an impeller o-ring.

[0044] It is not intended that the scope of the present disclosure is to be limited by the Examples described and illustrated above, but instead it is intended the scope of the present disclosure will be determined by the appended claims.

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- [WO2009009771A1 \[0005\]](#)
- [US2008131957A1 \[0005\]](#)

PATENTKRAV

1. Beholder til dyrkning af celler omfattende:
et beholderlegeme med en øvre del, en bunddel omfattende en indre bundoverflade og en cylindrisk sidevæg;
5 en hjulenhed inde i beholderlegemet omfattende en øvre del, der er roterbart koblet til den øvre del af beholderlegemet, hvilken hjulenhed har en flerhed af plane blade, en central akse, en fleksibel aksel, der strækker sig ned fra den øvre del af hjulenheden, et magnetleje støbt inde i flerheden af plane blade, en magnet inde i magnetlejet og en hjul-O-ring koblet til en bundoverflade af de plane blade; og
10 en flerhed af positioneringsknopper koblet til beholderlegemets indre bundoverflade, adskilt fra en indvendig kant af hjul-O-ringen.
2. Beholder ifølge krav 1, hvor flerheden af positioneringsknopper er tre positioneringsknopper, hver knop er periferisk placeret adskilt fra hver af de andre knopper i
15 omtrent samme afstand.
3. Beholder ifølge krav 2, hvor hver knop har en konisk form.
4. Beholder ifølge krav 2, hvor hver knop har en cylindrisk form.
20
5. Beholder ifølge et hvilket som helst af de foregående krav, hvor beholderlegemet omfatter et polymermateriale.
6. Beholder ifølge et hvilket som helst af de foregående krav, hvor flerheden af plane blade
25 er fire blade koblet til den fleksible aksel, hvor hvert blad er placeret i 90 grader i forhold til de tilstødende blade.
7. Beholder ifølge et hvilket som helst af de foregående krav, der endvidere omfatter:
en flerhed af baffler, hvor hver baffel, der er integreret med sidevæggen af
30 beholderlegemet, udgår fra den indre bundoverflade og strækker sig vertikalt til en forudbestemt afstand langs sidevæggen.
8. Beholder ifølge krav 7, hvor hver baffel afsluttes i en elliptisk form.

9. Beholder ifølge et hvilket som helst af kravene 7-8, hvor flerheden af baffler er tre baffler, hver baffel er placeret adskilt i samme afstand fra hver af de andre baffler omkring en perimeter defineret af den cylindriske sidevæg.
- 5 10. Beholder ifølge et hvilket som helst af de foregående krav, hvor beholderlegemet endvidere omfatter en radius målt ved afstanden fra den centrale akse til sidevæggen, og endvidere hvor mindst ét af bladene strækker sig ca. 50 til 95 % af beholderlegemets radius mod den cylindriske sidevæg.
- 10 11. Beholder ifølge et hvilket som helst af de foregående krav, hvor hvert blad omfatter en bundkant anbragt med en afstand på fra ca. 0,05 tomme til ca. 0,5 tomme fra den indre bundoverflade.
- 15 12. Beholder ifølge et hvilket som helst af de foregående krav, hvor hjulenheden er indesluttet inde i beholderlegemet.

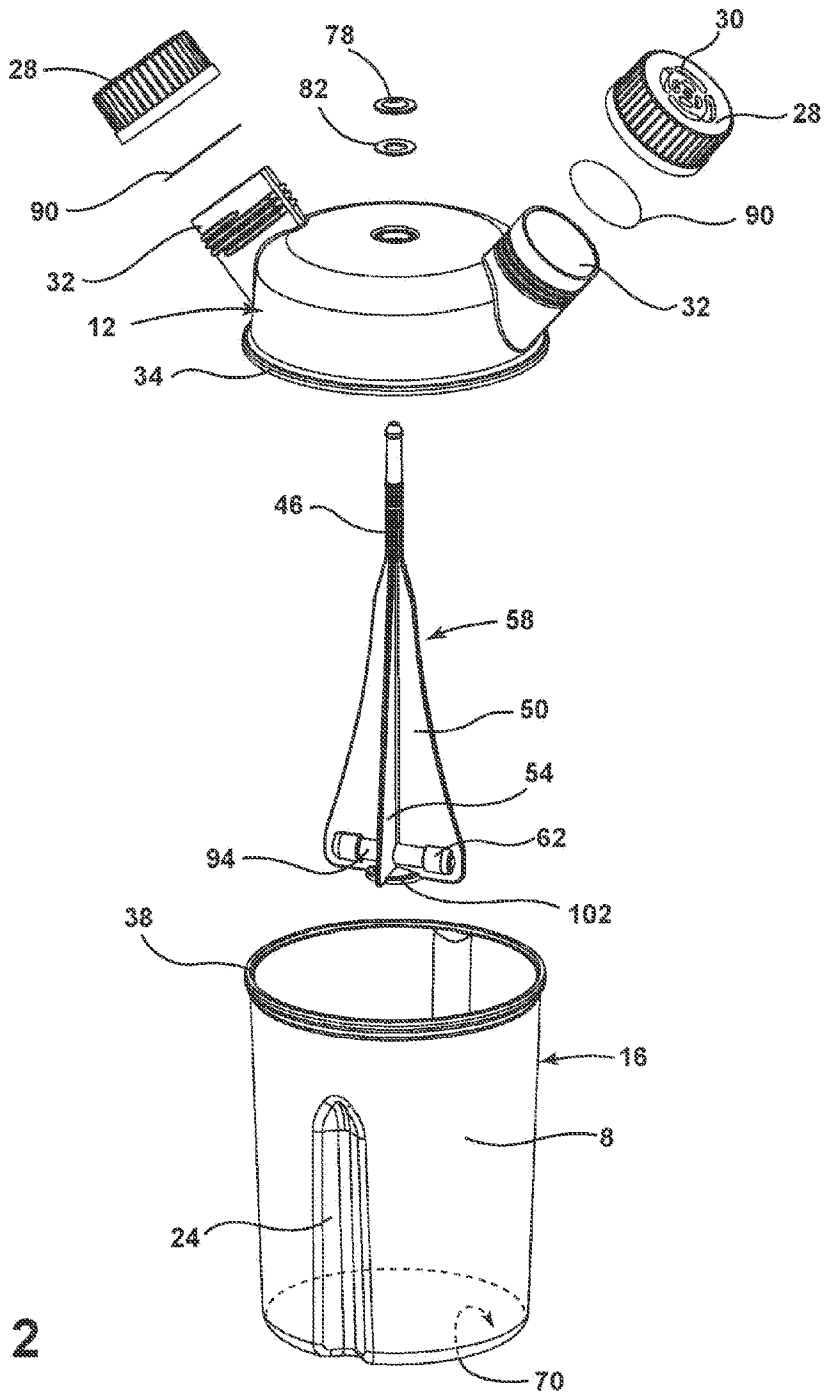


FIG. 2

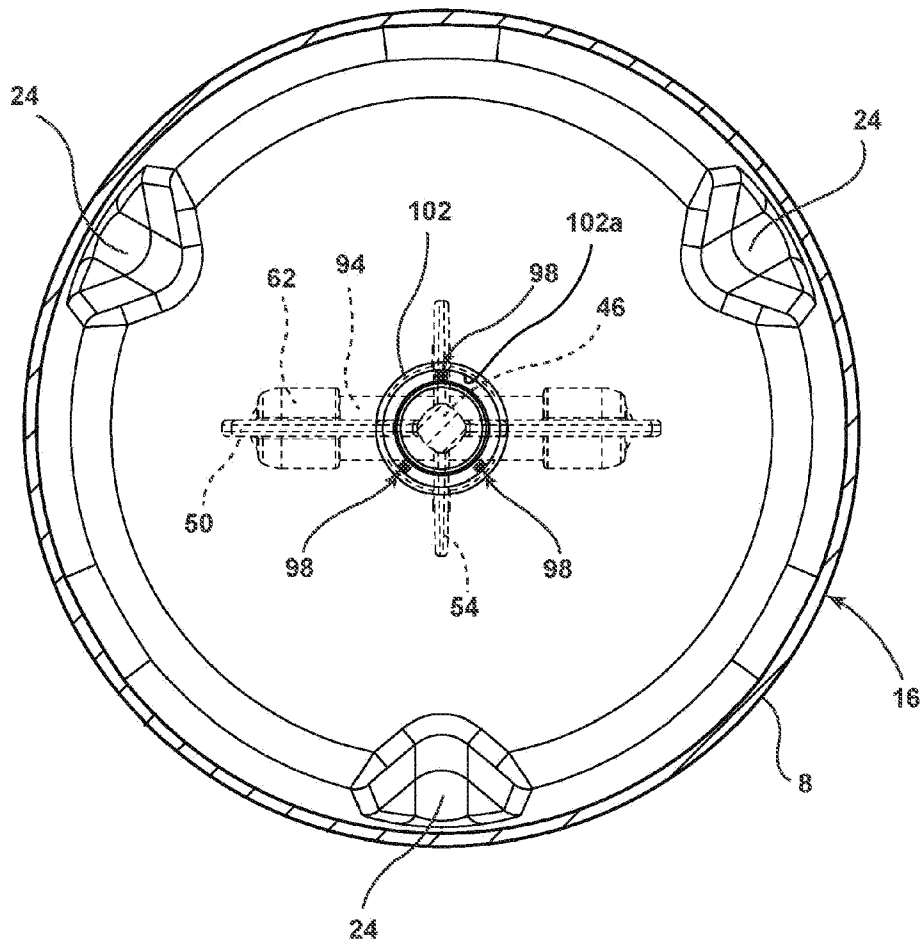


FIG. 3

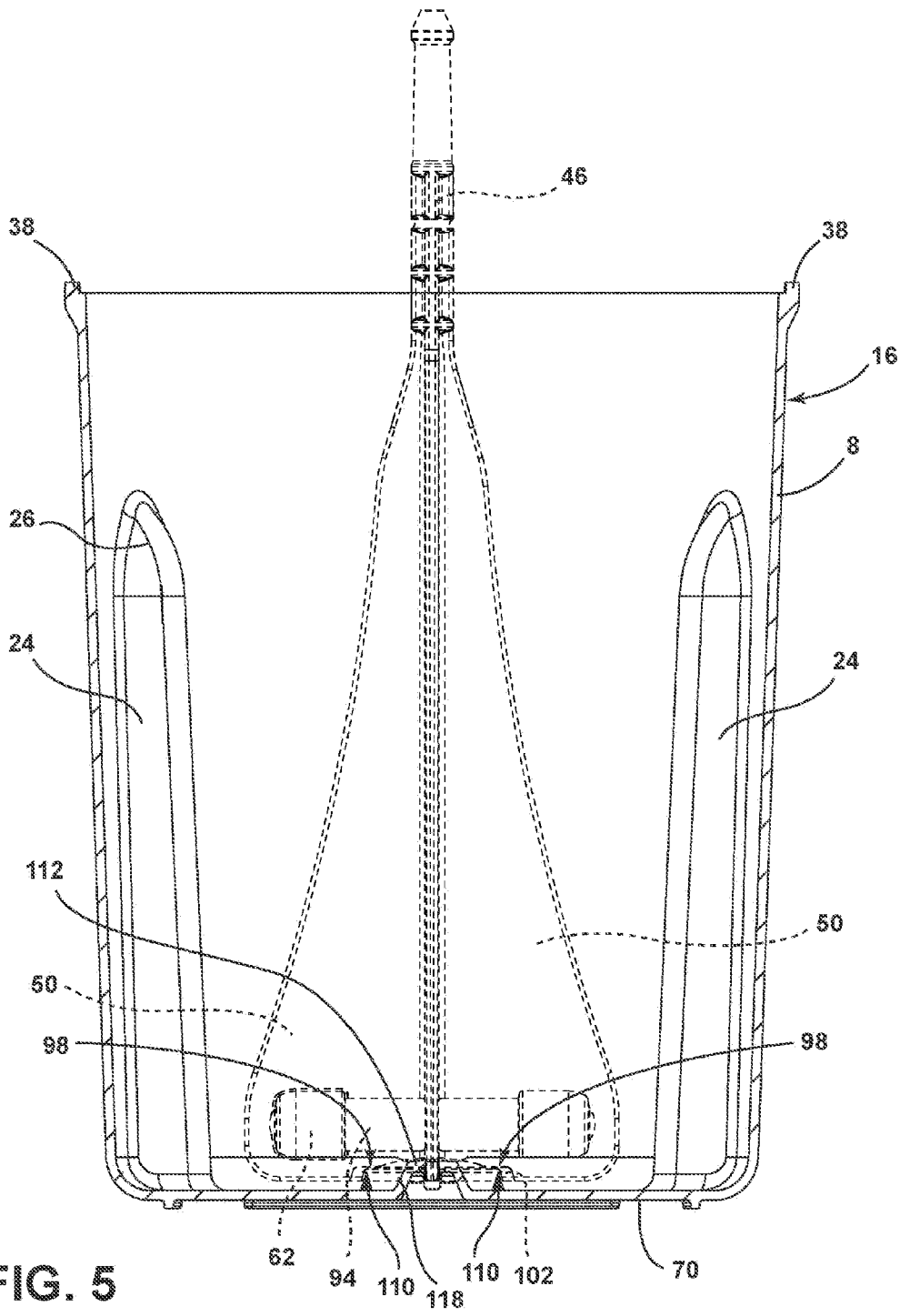


FIG. 5

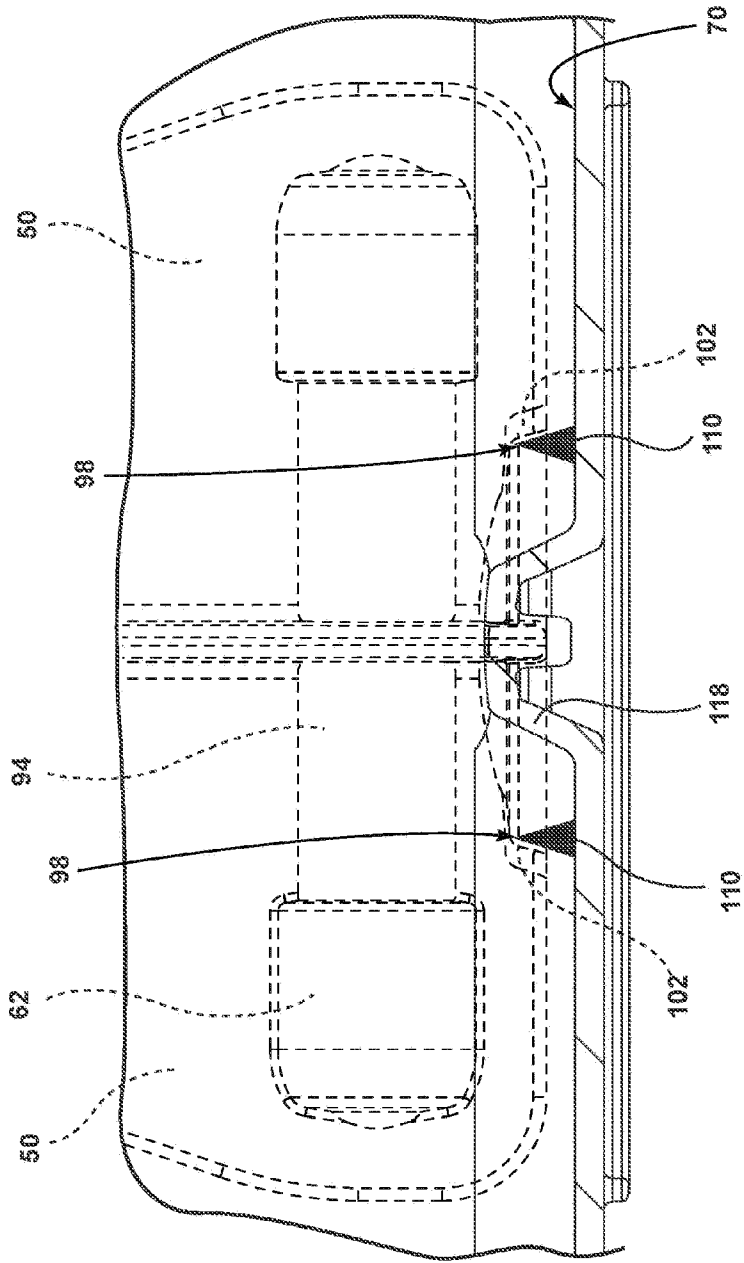


FIG. 6

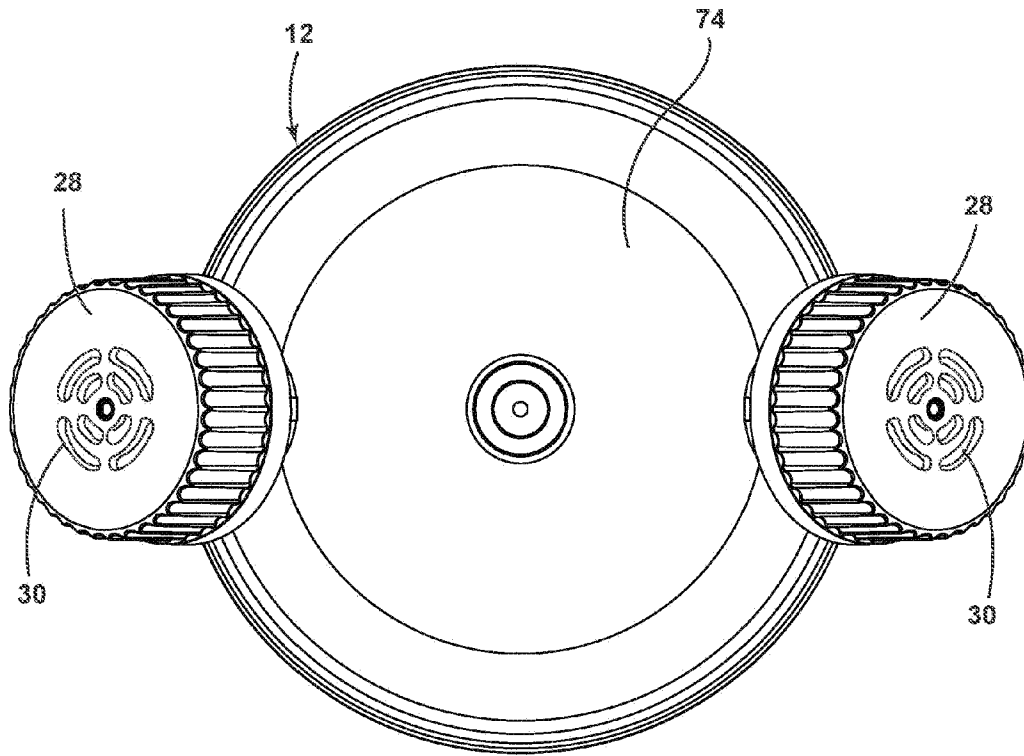


FIG. 7

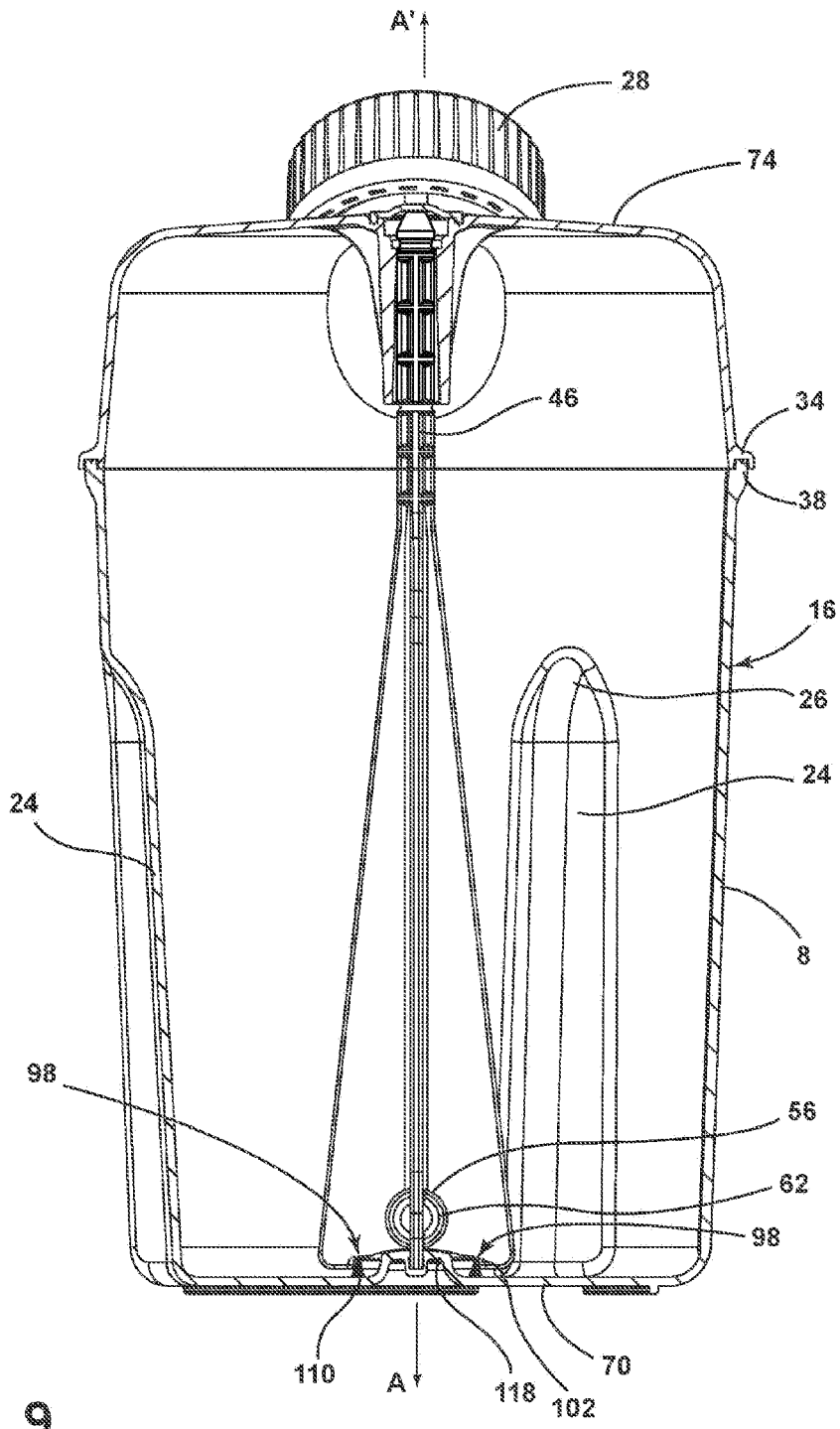


FIG. 9

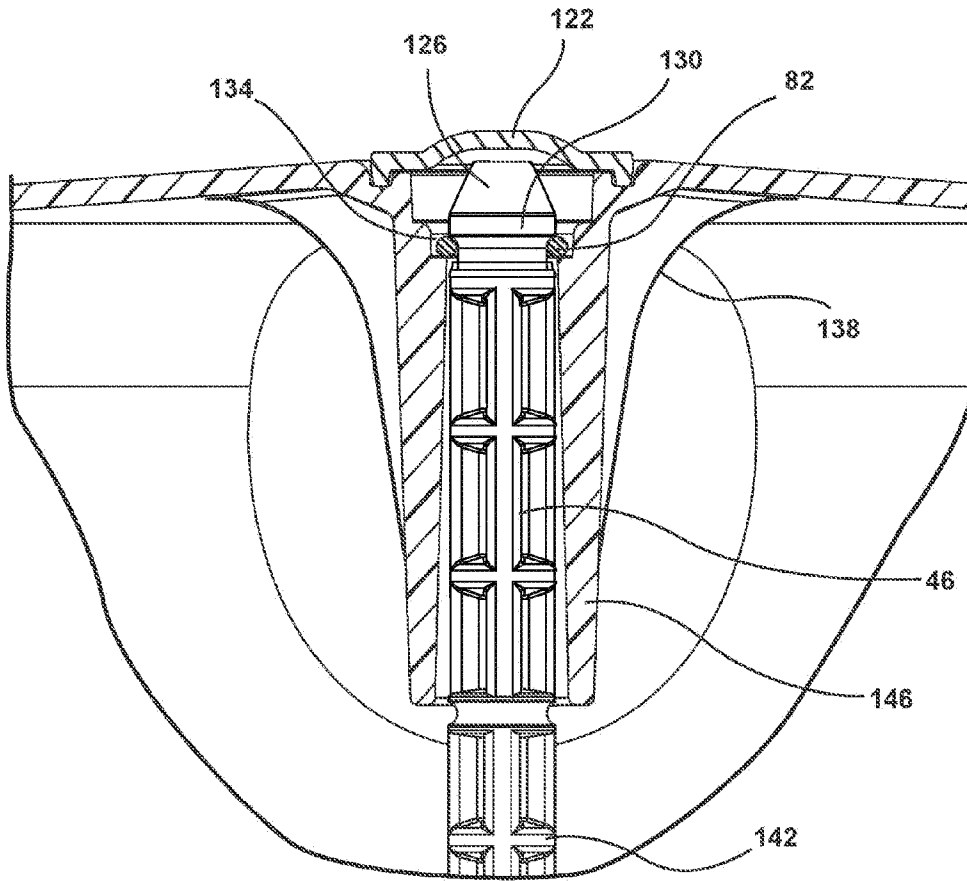


FIG. 10

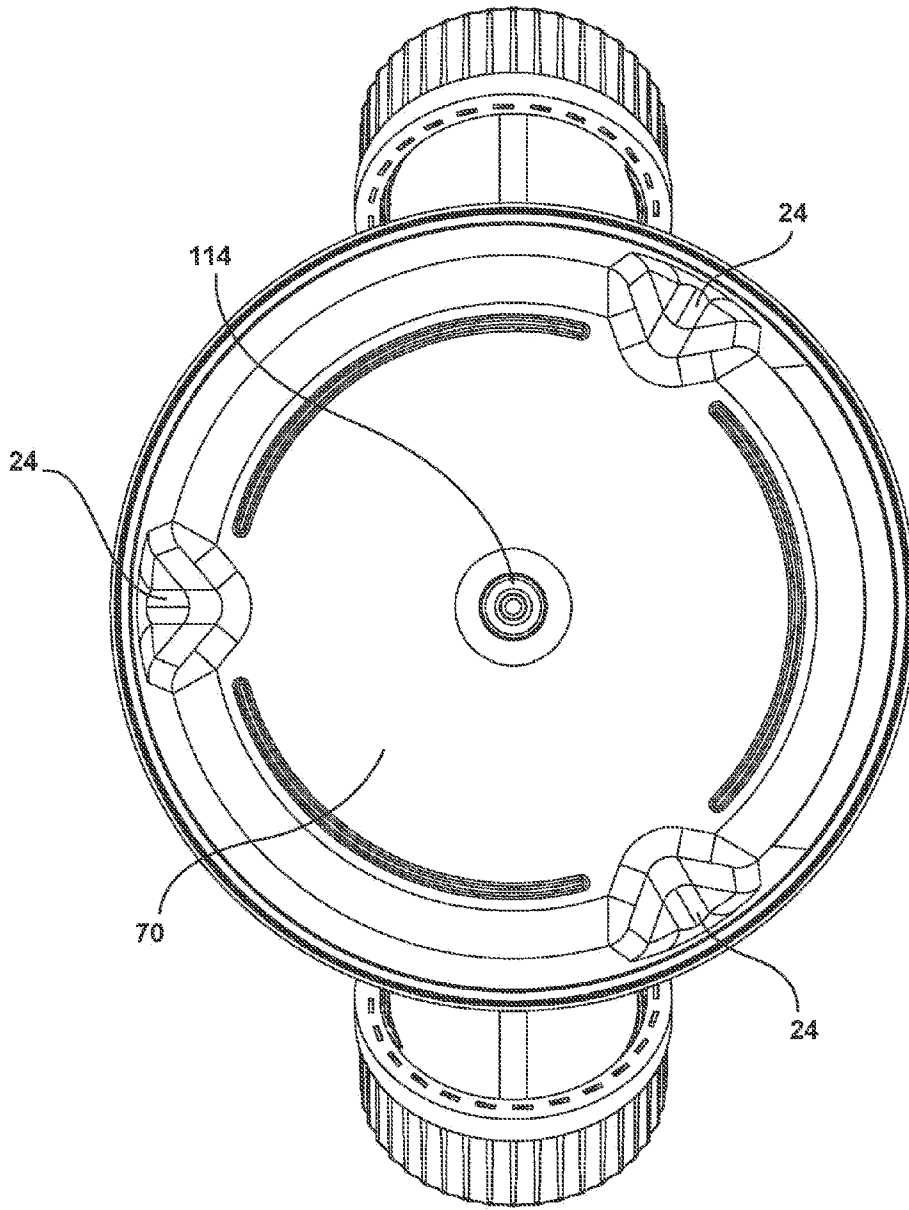


FIG. 11

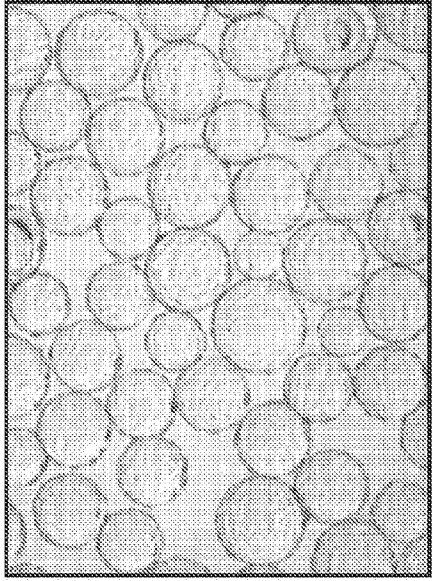


FIG. 12A

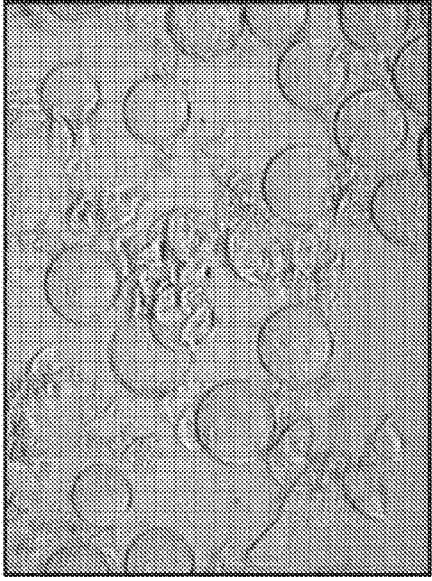


FIG. 12B

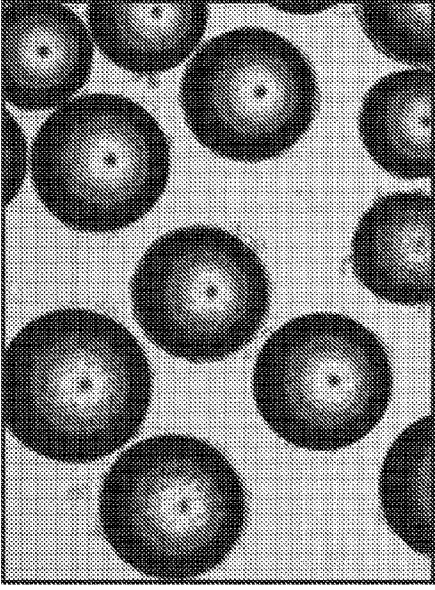


FIG. 13B

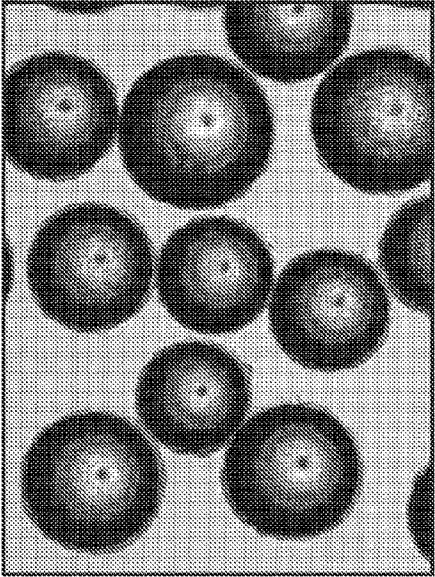


FIG. 13A

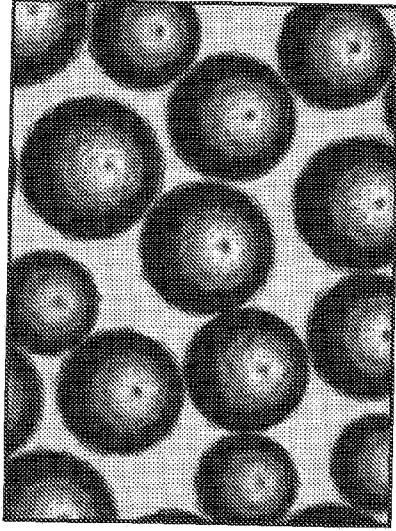


FIG. 14B

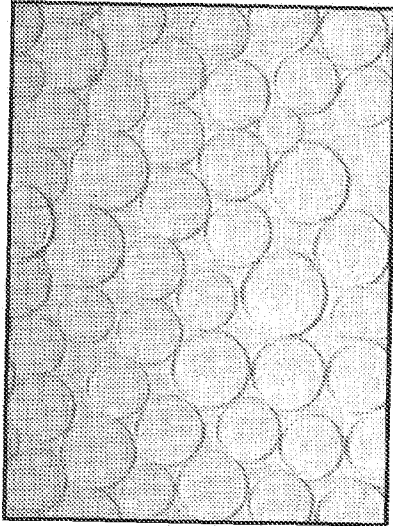


FIG. 14A