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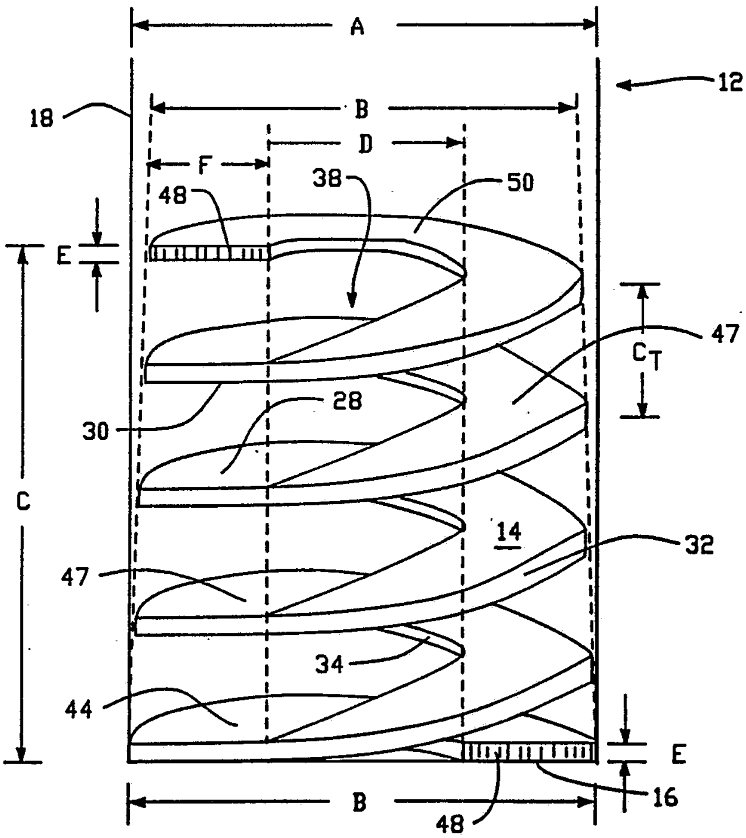
(54) Title: AN APPARATUS FOR USE IN CHEMICAL, ENZYMATIC AND IMMUNOASSAY REACTIONS

(57) Abstract

An apparatus for use in chemical, enzymatic and immunoassay reactions is disclosed which comprises a vessel (12), a reaction fluid in the vessel and a spiraling support (14) with coils inserted in the vessel. Each coil has a top surface (28), a lower surface (30), an outwardly facing surface (32) and an inwardly facing surface (34). The surfaces are adapted to associate with a reactant and the coils are constructed so that the fluid is freely flowable over substantial portions of the coil surfaces. Use of the support in conjunction with the vessel increases the reaction rate from about 8 to 50 fold over the reaction rate which occurs without the support.



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AN APPARATUS FOR USE IN CHEMICAL, ENZYMATIC AND IMMUNOASSAY REACTIONS

INVENTOR:
Marie Mei-Yi Tcheng Lin

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to an apparatus for use in chemical, enzymatic and immunoassay reactions and more specifically to an apparatus including a support for increasing the reactive surface area available during such reactions.

2. Background

The use of supports as carriers of various reactants in enzymatic assays, immunodiagnostic tests and affinity purifications is well known. This is because current enzyme and immunoassays, especially those performed in microtiter plates, suffer slow kinetics and relatively low sensitivity due to the limited sample size and surface area of the vessel. Examples of such carriers are disclosed in U.S. Patent Nos. 4,111,074; 4,147,752; 4,197,287; 4,225,575; 4,495,151 and the article by Ferrua et al. entitled, "A Novel Enzyme Immunoassay For Total Thyroxine Using Immobilized Antibodies and Hydrophobic Chromatography Purified Thyroxine-Peroxidase Conjugate," J. Immuno. Meth. 87: 137-143 (1986).

As is often the case, supports which provide a sufficient increase of reactive surface area usually have other disadvantages in that it is difficult to insure the desired mobility of the fluid or the fluid's complete removal when required. This is especially the case for reactions occurring in microtiter type vessels. The support device disclosed in U.S. Patent No. 4,111,754, for example, provides an increased surface area but does not provide for the free circulation of a reaction fluid over that area. Also, because of numerous narrow, discontinuous grooves and cavities, the complete and efficient removal of fluid from a vessel containing such a support is severely inhibited. The ring or cylinder disclosed in U.S. Patent No. 4,147,752 allows for relatively free circulation of the fluid but does not provide for a very significant increase in surface area. U.S. Patent No. 4,197,287 discloses carriers with an acceptable increase in surface area but again the existence of tight corners with respect to the floor of the vessel and a small diameter to length ratio make the removal and circulation of fluid extremely difficult even with automatic pipetting equipment. U.S. Patent Nos. 4,225,575 and 4,495,151 disclose carriers which cannot, without added support, remain upright in a flat bottom vessel, are particularly inapplicable to microtiter dishes and which have to be removed from the vessel prior to the measurement of any resulting reaction. The carrier disclosed in the Ferrua reaction must also be removed prior to any measurement determination.

<u>SUMMARY OF INVENTION</u>

The present invention is designed to overcome such problems. One aspect of the invention is to provide a reaction apparatus that comprises a vessel, a reaction fluid located in the vessel and a spiraling support inserted in the fluid in the vessel during the reaction. The vessel may be any type of laboratory vessel such as a test tube, a cuvette, or a microtiter well. The support has an outer coil which extends full circle and at least one inner coil which extends full circle. Each coil has a top surface, a lower surface, an outwardly facing surface, and an inwardly facing surface, whereby at least one of the inwardly facing surfaces defines at least a portion of a cavity and all of the surfaces are adapted to associate with a reactant, and the coils are constructed so that the fluid is freely flowable over substantial portions of the inwardly facing surfaces, the top surfaces, and said outwardly facing surface of each inner coil.

Another aspect of the invention is to provide an apparatus that includes a vessel, a reaction fluid and a spiraling support where the support has a bottom coil which extends full circle and at least one upper coil with each coil having a top surface, a lower surface, an outwardly facing surface and an inwardly facing surface. Again, all the surfaces are adapted to associate with a reactant and at least one of the inwardly facing surfaces defines at least a portion of a cavity and the coils are so constructed with respect to each other and the vessel that the fluid is freely flowable over substantial portions of the surfaces.

The supports, while always spiral, may have various shapes which may be substantially cylindrical, disk-like or helical with equal sized coils or the shape may be that of a spiraling ramp with progressively sized coils.

A third aspect of the invention is to have the above-described supports elastically compressible and to have a substantial portion of the outwardly facing surface of the outer coil or bottom coil pressed tightly against the vessel so that the support will not fall if the vessel is shaken or turned upside down during normal use.

A fourth aspect of the invention is for each of the above-described supports to be a spring which may be temporarily compressed.

Yet another aspect of the invention is for the cavity of the above-described supports to include a center through portion adapted to accept at least a part of an aspirating or pipetting device.

Still a further aspect of the invention is for the lower surfaces of each of the outer coil to be substantially flat and for the spring to be substantially helical. Still another aspect of the invention is to make the support seamless and integrally injection molded.

A further aspect of the invention is a support for use in a reaction fluid in a vessel during a reaction where the support comprises a helical, spring. The support has an outer coil and at least one inner coil and each coil has an inwardly facing surface, an outwardly facing surface, a top surface and a lower surface, whereby the inwardly facing surfaces define a center through cavity into which at least a part of an aspirating or pipetting device may be inserted. All of the surfaces are adapted to associate with a reactant in the reaction and the coils are constructed so that the fluid is freely flowable over substantial portions of the inwardly facing surfaces, over all the top surfaces and over the lower surface of each inner coil. Still another aspect of the invention is to have the outwardly facing surface of the outer coil of the support pressed tightly against the vessel so that the support will not fall if the vessel is shaken or turned upside down during normal use.

Yet still a further aspect of the invention is a helical spring support, with equally sized coils whereby the support has a bottom coil and at least one upper coil.

The reaction apparatus and support may be used in immunological, chemical and enzymatic procedures. A major aspect of the reaction apparatus and, specifically, the support is to increase the reaction kinetics by immobilizing and increasing the attachment of reactants, such as antigens, antibodies and enzymes, to the available surface relative to the total volume of fluid in the reaction, thereby permitting less fluid, while enhancing the intensity and accuracy of the results of the reaction. This increases the surface to volume ratio of the reaction and decreases the distance between the attached reactants in the reaction in relation to the mobile reactants.

The reaction apparatus may be used, for example, in detecting the presence or determining the amount of a particular antibody in a reaction fluid. The support is set in a reaction vessel such as a microtiter well and a solution containing the appropriate reactant to be attached, in this instance an antigen, is added to the vessel. After the antigen has been allowed to associate with the surfaces of the support and the inner surfaces of the vessel, the solution is removed and the support and vessel are washed so as to remove only any extraneous, i.e. non-attached, antigens. A biological reaction fluid which may or may not contain the corresponding antibody is then added to the vessel. If the appropriate antibodies are present they will attach to the immobilized antigens. Following significant exposure the biological fluid is removed and the vessel and support are again carefully washed with an appropriate solution. After removal of the washing solution, another reaction fluid containing an antibody enzyme conjugate and usually a buffer is introduced into the well. The antibody in this second reaction fluid corresponds to the antibody being determined. Again, after an appropriate incubation period, the second reaction fluid is removed and the vessel and support are carefully washed. An indicator substrate is then added to the vessel and the presence of the antibody as well as the amount of antibody in the biological fluid can be determined by either a color change or a kinetic or end-point measurement.

The support may be made of glass, cellulose or a plastic or polymeric material, such as polyvinyl polycarbonate, polyacetate or polystyrene, preferably polystyrene, or of other material to which the immobilized reactant will associate either by covalent binding, adsorption or some other means known in the prior art.

Still another aspect of the invention is to have the coils of such a size and shape in relation to each other and the vessel (i.e. so constructed) so that the fluid will flow freely over almost all of the support. The number of coils may vary, but about 3-6 coils are preferable.

In the preferred embodiments, the fluid will flow freely over all the coil surfaces that are not pressed tightly against (i.e. contacting) the vessel and therefore drain freely either by aspiration or decantation without removal of the support from the vessel. This drainage will occur even when microtiter type vessels and microtiter volumes are used.

Still another aspect of the invention, when a transparent vessel is used, is for the center through cavity of the support to permit the through passage of a photometric beam so that the reaction results may be detected or quantitatively or qualitatively analyzed without the removal of the support.

In another embodiment, the support is a flexible spring comprising a series of coils which allows for washing and mixing of the reaction fluid by merely pressing the support down with, for example, the tip of a pipet and then releasing the spring by lifting the tip up. In a further aspect of the invention, the bottom, or outer coil, may be constructed so as to conform to the bottom of the vessel and, therefore, provide intimate contact with the vessel. Because of the increased surface to volume ratio, and decreased distance between the reactants in the reaction, a further aspect of the invention is to construct the support coils so that at least about 50 to 100 percent of the support surfaces are in contact with the reaction fluid and the reaction rate is increased from about 8 to 50 fold. This also increases the reliability of the reaction and quantitative analysis can easily be performed in a reaction volume of only a few cubic millimeters.

Other aspects and advantages of the invention are discussed further in the following detailed description of the embodiments, in conjunction with the accompanying drawings which show the preferred and various other embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG.1 is a side perspective view of the reaction apparatus showing a top and side perspective view of an embodiment of the support in cylindrical form.

FIG. 2 is a side perspective view of the reaction apparatus without the fluid showing a top and perspective view of another embodiment of the support in disk form.

FIG. 3 is a side elevational view of the reaction apparatus showing an exemplifying embodiment of the support.

FIG. 4 is a side elevational view of the reaction apparatus showing an alternative embodiment of the support.

DETAILED DESCRIPTION OF THE INVENTION

Referring now to FIG. 1, there is seen a reaction apparatus 10 for use in a solid phase system and other reactions having a reaction vessel 12, a reaction fluid 13 located in the vessel 12 and an elastic, spiraling support 14 inserted in the fluid 13 in the vessel 12. The support 14 is made from an elastic, resilient material, such as certain types of polymeric materials, preferably polystyrene. The support may also be rigid and made from other materials such as glass or rigid plastics. The vessel 12, as in this instance, is preferably transparent and made of clear glass or plastic, but it may also be made from opaque materials. The vessel 12 has a floor 16 and a wall 18. The support 14 has an outer coil 24, which extends full circle and inner coils 26, including two inner coils 26, which extend full circle, as well as the beginnings of a third inner coil 26. Each coil 24, 26 has a top surface 28, a lower surface 30, an outwardly facing surface 32 and an inwardly facing surface 34. The surfaces 28, 30, 32, 34 are adapted to associate with reactants, such as hormones, enzymes, drugs, antibodies, antigens, metabolites and other polyamino acids and carbohydrates. The reaction apparatus 10 can be used in immunoassays such as enzyme immunoassays (EIA), radioimmunoassays (RIA), or other assays where a biological fluid is used. The inwardly facing surfaces 34 of the support 14 define a cavity 36 which spirals throughout the support 14 and extends from the top surfaces 28 to the lower surfaces

30. All the coils 24, 26 are shaped and spaced in relation to each other and the vessel so that the fluid 13 is freely flowable and freely circulates over all of the top surfaces 28, the lower surfaces 30, the outwardly facing surfaces 32 of the inner coils 26 and the inwardly facing surfaces 34 of the outer coil 24 and the inner coils 26. The cavity 36 includes a center through cavity 38 which is adapted to accept at least part of an aspirating or pipetting device, such as the tip of an aspirator or a pipette, thereby enabling an investigator to place the tip of an aspirator on the floor of the vessel 16 and aspirate the entire fluid 13 without removing the support 14. The center through portion 38 also allows for the passage of a photometric beam through the apparatus 10 so that the reaction may be detected or quantitatively or qualitatively analyzed by an instrument such as a spectrophotometer without the removal of the support 14 from the vessel 12. FIG. 1 shows the apparatus in position for vertical measurement (i.e. the beam will pass through the central through portion 38 in the direction of the longitudinal axis of the vessel 12). If, however, it is necessary for the beam to pass horizontally through the horizontal axis of the vessel 12, the support 14 may still be used by turning the support 14 on its side so that the center through cavity 38 is perpendicular to the longitudinal axis of the vessel 12. The support 14, as depicted in FIG. 1, is substantially cylindrical and seamless and integrally molded. Also, the outwardly facing surface 32 of the outer coil 24, shown in FIG. 1, is pressed sufficiently tightly against the vessel 12 so that the support 14 will not fall out of the vessel 12 if the vessel 12 is shaken or turned upside down for decantation during normal use. The cavity 36 of the vessel 12 is also large enough so that the fluid 13 will not adhere to the inwardly facing surfaces 34 and outwardly facing surfaces 32 of the inner coils 26 or the vessel floor

16 or wall 18 or to any exposed portion of the support 14 when the vessel 12 is decanted, thereby allowing for free drainage of the fluid 13 and any other liquid. This same attribute ensures that no fluid pockets or corners are formed and the fluid 13 and any other liquid circulates freely over all of the top surfaces 28, lower surfaces 30, inwardly facing surfaces 34 and the outwardly facing surfaces of the inner coils 26. Since all the surfaces 28, 30, 32, and 34 are adapted to associate with a reactant, the reaction rate of the reaction is greatly enhanced and small reaction fluid volumes may be used. The embodiment depicted in FIG. 2 shows a reaction vessel 12 disk-like version of the support 14 described above as depicted in FIG. 1.

The embodiments depicted in FIGS. 3 and 4 are used in the same manner as the manner described for the embodiments depicted in FIGS. 1 and 2. In the embodiments depicted in FIGS. 3 and 4, a reaction vessel 12 is depicted, with a substantially helical spiralling support 14 inserted in the vessel 12. The reaction fluid 13 depicted in FIG. 1 is not shown in FIGS. 2-4. The support 14 has a bottom coil 44 and three and one half upper coils 47. Each coil 44, 47 has a top surface 28, a lower surface 30, an outwardly facing surface 32, and in inwardly facing surface 34. Again, the surfaces are adapted to associate with reactants. The inwardly facing surfaces 34 make up a center through cavity 38 which, as in the embodiments depicted in FIGS. 1 and 2, may be used for the insertion of at least a portion of a pipetting or aspirating device as well as for the passage of a photometric beam for purposes of analyzing the reaction without removing the support 14. While all the figures depict a support 14 with a central through portion 38, embodiments of a support 14 without such a through portion 38 may also be used. For example, the top coil 50 shown in FIGS. 3-4 could be a solid disc without an opening. The support 14, as shown in FIGS. 3 and 4, is a flexible, flat spring made of a polymeric material, such as polystyrene, which is temporarily, longitudinally compressible. The temporary compression of the support 14 and relief of that compression provides a means for effectively mixing the fluid 13 when it is in the reaction vessel 12. The pressure may be applied, for example, by pressing down the top surface 28 of the top coil 50 with, for example, a pipette tip and releasing the pressure by lifting up the pipette tip. The supports 14 shown in FIGS. 3-4 are also integrally molded and seamless. Again, the outwardly facing edge 32 of the bottom coil 44, fits against the vessel 12 so that the support 14 will not slip or fall if the vessel 12 is turned upside down for decantation during normal use. Also, the fluid 13 will flow freely over and drain freely from all the top surfaces 28, inwardly facing surfaces 34 and from the lower surfaces 30 and outwardly facing surfaces 32 of all the upper coils 48 of the support 14 depicted in FIG. 3. In the support 14 in FIG. 4 the fluid 13 will also flow freely over the lower surface 30 of the bottom coil 44. In FIGS. 3 and 4 the fluid 13 and any other liquid will drain freely from the vessel 12 when desired without removal of the support 14. In FIG. 4, the bottom coil 44 is also the outermost coil 24 and the upper coils 47 are also the inner coils 26. However, the coils in FIG. 3 are all the same size, so there are no inner 26 or outer 24 coils.

Referring now to FIG. 3, there is shown a preferred embodiment of the support 14 particularly adapted for use with a microtiter plate. If the vessel 12 is a microtiter well, which has a top inside diameter of 6.8 mm, shown as A, then the outside diameter of each coil 44, 47, shown as B, is optimally 6.3 mm. Note that microtiter wells manufactured by molding are conventionally wider at the top than at the bottom. The height of the support 14, shown as C, is 6.0 mm (2.5 mm when compressed) when the support 14 is fully released. The diameter of the center through cavity 38 is 3.0 mm, shown as D. Also shown in FIG. 3 is a cross-section 48 of the coils 44, 47. The height of the cross-section 48, shown as E, is preferably 0.4 mm or somewhat less and the length of the cross-section 48, shown as F, is 1.5 mm. The distance between the middle of the height E of the cross-section 48 of the coil 47 to the middle of the height of the next cross-section E of the next coil 47 of the support 14, shown as G, is 1.5 mm when the support 14 is fully released. The distance between the middle of the height E of the cross-section 48 of the bottom coil 44 to the middle of the height of the next cross-section E of the next coil 47 of the support 4 is, of course, also 1.5 mm when the support 14 is not compressed downward. Assuming that the support 14 is fully immersed in the reaction fluid 13 and the lower surface 30 of the bottom coil 44 is exposed as shown in FIG. 4, the increased reaction rate which will result from using a support 14 having these dimensions can be calculated as follows:

1. Vessel Surface (S_2)

$= 2\pi r \cdot 6 + \pi r^2$ where $2r = 6.5$ mm

$= 122 + 31$

$= 153$ (mm^2)

2. Support Top and Lower Surfaces (2 sides)

$= (\pi r_1^2 - \pi r_2^2)\ 4.5 \cdot 2$ where $r_1 = 3.1$ mm

$= \pi\ (3.1^2 - 1.5^2) \cdot 4.5 \cdot 2$ $r_2 = 1.5$ mm

$= 104 \cdot 2$

$= 208$ (mm^2)

3. Support, Outwardly and Inwardly facing surfaces (2 edges)

$= 0.4 \cdot 2\pi r_1 \cdot 4.5 + 0.4 \cdot 2\pi r_2 \cdot 4.5$

$$= 0.4 \cdot 4.5 \cdot 2\pi(r_1 + r_2)$$
$$= 52 \ (mm^2)$$

4. Total Surface Area, Vessel (S_2) and Support (S_1)

$$= 153 + 208 + 52$$
$$= 413 \ mm^2$$

5. R (reaction rate) = S (surface area) $\cdot \left[\frac{1}{d}\right]^2$

where d = distance between two reactants

S_1 (vessel + support) = 413 mm^2 d_1 = 0.75mm

S_2 (vessel alone) = 153 mm^2 d_2 = 3.25mm

$$\frac{R_1}{R_2} = \frac{S_1}{S_2} \cdot \left[\frac{d_2}{d_1}\right]^2$$

$$\frac{\text{Rate (with support)}}{\text{Rate (no support)}} \approx 51$$

Therefore, using the vessel and support depicted in FIG. 3, the surface area is increased from about 153 mm^2 to about 413 mm^2 thereby decreasing the distance between two reactants from 3.25 mm to 0.75 mm, and increasing the reaction rate approximately 51 fold.

Two major factors contribute to reaction kinetics, the total surface area and the distance between the two reactants, such as the distance between an enzyme and a substrate. A reaction rate is directly proportional to the surface area, and is reversely proportional to the square of the distance between two reactants. Approximately 190 microliters are needed to cover the whole support 14 shown in FIG. 3, however, it is not necessary to use the entire support. From 50 to 400 microliters, preferably 200 to 300 microliters will enable the apparatus of the invention and the support to effectively increase the reaction kinetics in a microtiter well.

Although the foregoing invention has been described in some detail by way of illustrations, it should be realized that various modifications, as may be apparent to one of skill in the art to which the invention pertains, may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A reaction apparatus comprising:

1) a vessel;

2) a reaction fluid located in said vessel; and

3) a spiraling support, inserted in said fluid in said vessel, said support having an outer coil which extends full circle, and at least one inner coil which extends full circle, each said coil having a top surface, a lower surface, an outwardly facing surface and an inwardly facing surface, whereby at least one of said inwardly facing surfaces defines at least a portion of a cavity, said surfaces being adapted to associate with a reactant and said coils being constructed so that said fluid is freely flowable over all substantial portions of said inwardly facing surfaces, said top surfaces and said outwardly facing surface of each said inner coil.

2. The apparatus of claim 1 wherein the support is a substantially cylindrical, spiraling support.

3. The apparatus of claim 1 wherein said support is a disk-like, spiraling support.

4. The apparatus of claim 1 wherein said support is a spiraling ramp.

5. The apparatus of claim 1 whereby said support is elastically compressible and a substantial portion of said outwardly facing surface of said outer coil is pressed sufficiently tightly against said vessel so that the support will not fall out of said vessel if said vessel is shaken or turned upside down during normal use.

6. The apparatus of claim 5 wherein the support is a spring.

7. The apparatus of claim 1 wherein said support cavity defined at least partially by said inwardly facing surfaces includes a center through portion adapted to accept at least a part of an aspirating or pipetting device.

8. The apparatus of claim 7 whereby said vessel is transparent and said center through portion allows for the passage of a photometric beam through the apparatus so that the reaction may be detected or quantitatively or qualitatively analyzed by an instrument without the removal of the support from the vessel.

9. The apparatus of claim 7 wherein said support is a spring which may be temporarily longitudinally compressed whereby said temporary compression of said support and the relief of that compression provide a means for effectively mixing the fluid.

10. The apparatus of claim 7 wherein the vessel is a microtiter well and the reaction fluid has a volume of about 0.2-0.35 ml.

11. A support for insertion and use in a reaction fluid in a vessel during a reaction whereby said reaction has a first reaction rate without use of said support and a second reaction rate with the use of said support, said support comprising:

an elastic, substantially helical, spring, said spring having at least one bottom outer coil and at least one upper coil, each said coil having an inwardly facing surface, an outwardly facing surface, a top surface and a lower surface, said surfaces being adapted to associate with a reactant in the reaction, whereby said inwardly facing surfaces define a center through cavity into which at least a part of an aspirating or pipetting device may be inserted, and said coils are constructed so that said fluid is freely flowable over substantial portions of said inwardly facing surfaces, said top surfaces and over a substantial portion of said lower surface of each said inner coil when said support is inserted in said fluid.

12. The support of claim 11 whereby said fluid is freely flowable over said outwardly facing surface of each said upper coil when said support is inserted in said fluid.

13. The support of claim 11 whereby a substantial portion of said outwardly facing surface of said bottom coil is pressed sufficiently tightly against the vessel so that said support will not fall out of said vessel if said vessel is shaken or turned upside down during normal use.

14. The support of claim 13 whereby said support may be temporarily longitudinally compressed by the application of an outside force, whereby said temporary compression and the relief of said compression provide a means for effectively mixing the fluid.

15. The support of claim 13 whereby the support is of a construction such that at least about 50 to 100 percent of its surfaces are in contact with said reaction fluid and the reaction rate is increased from about 8 to 50 fold when said support is inserted in said reaction fluid in a vessel during said reaction.

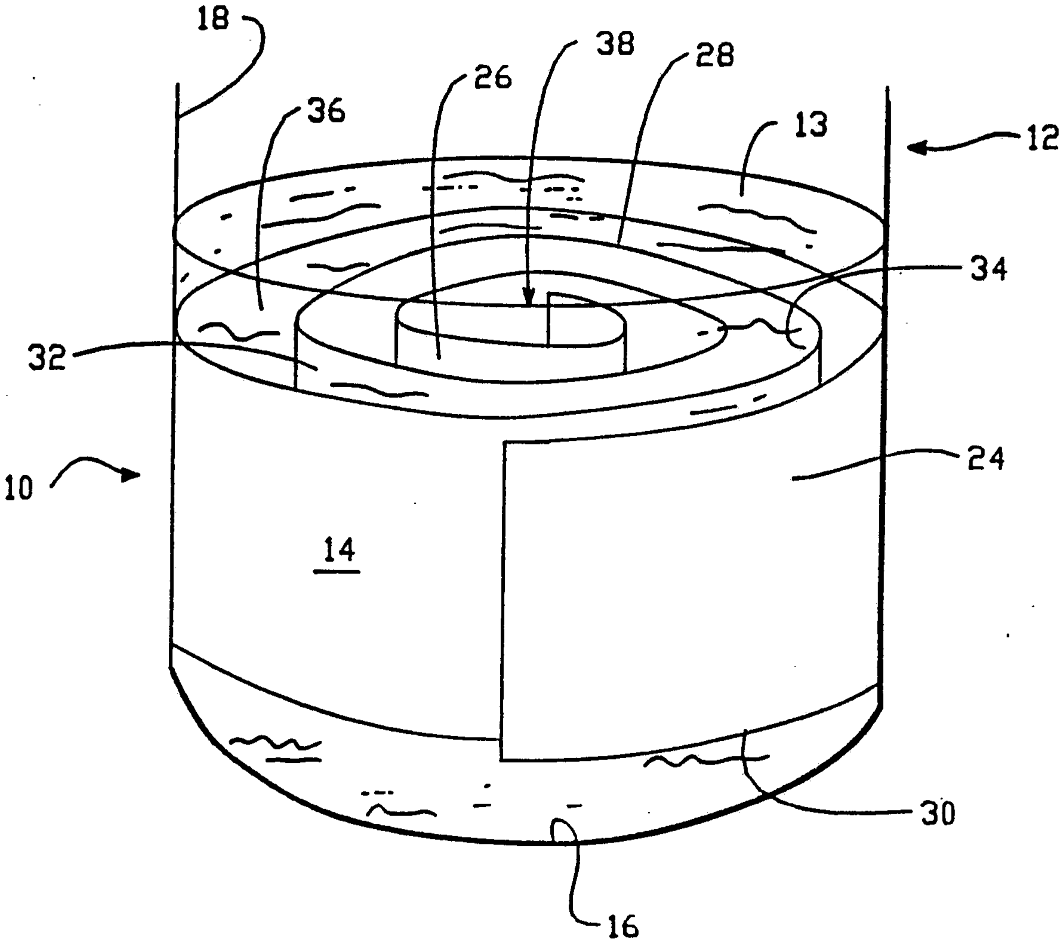

FIG.−1

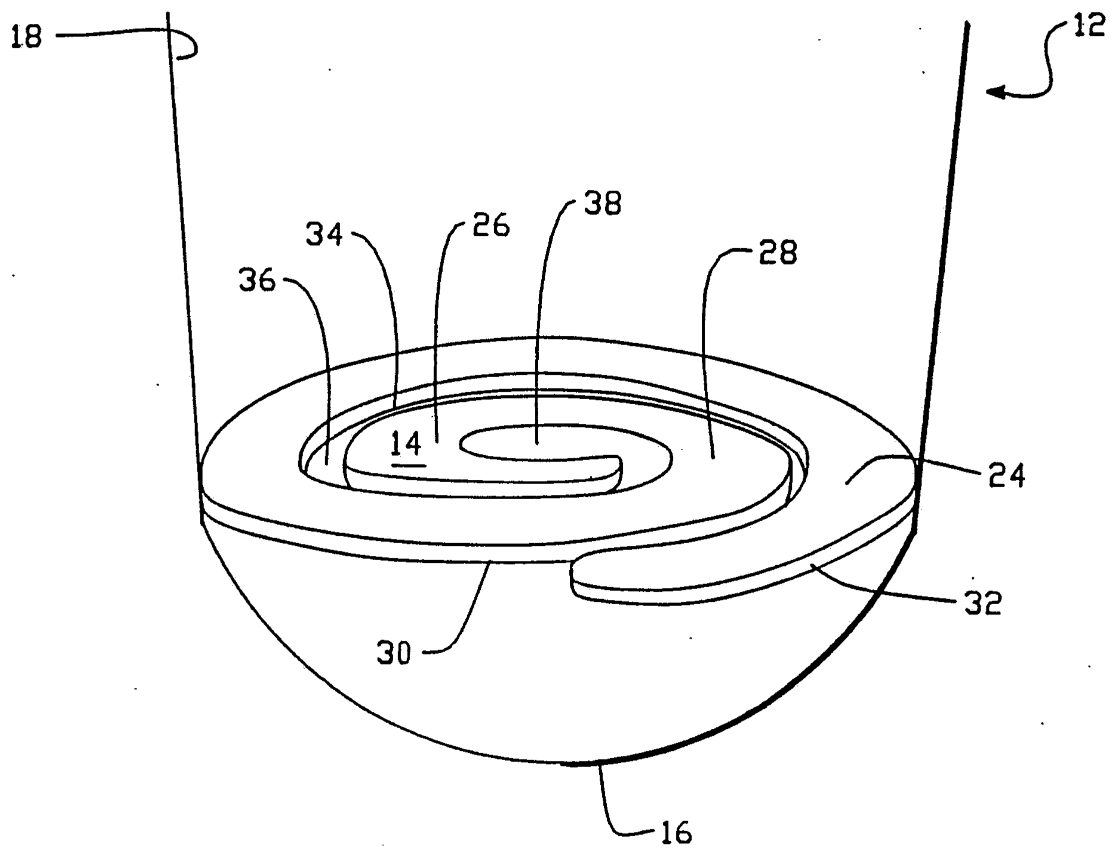

FIG.-2

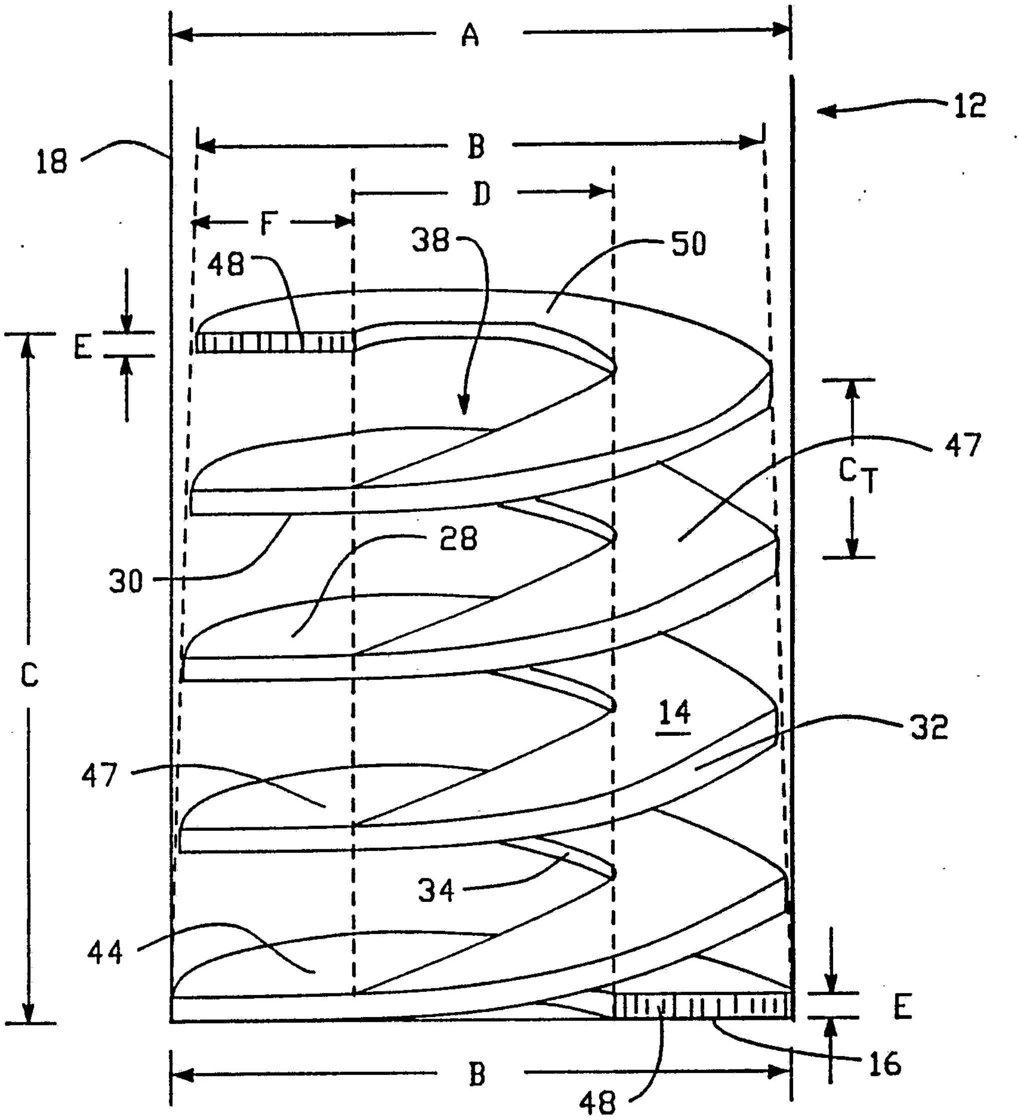

FIG.—3

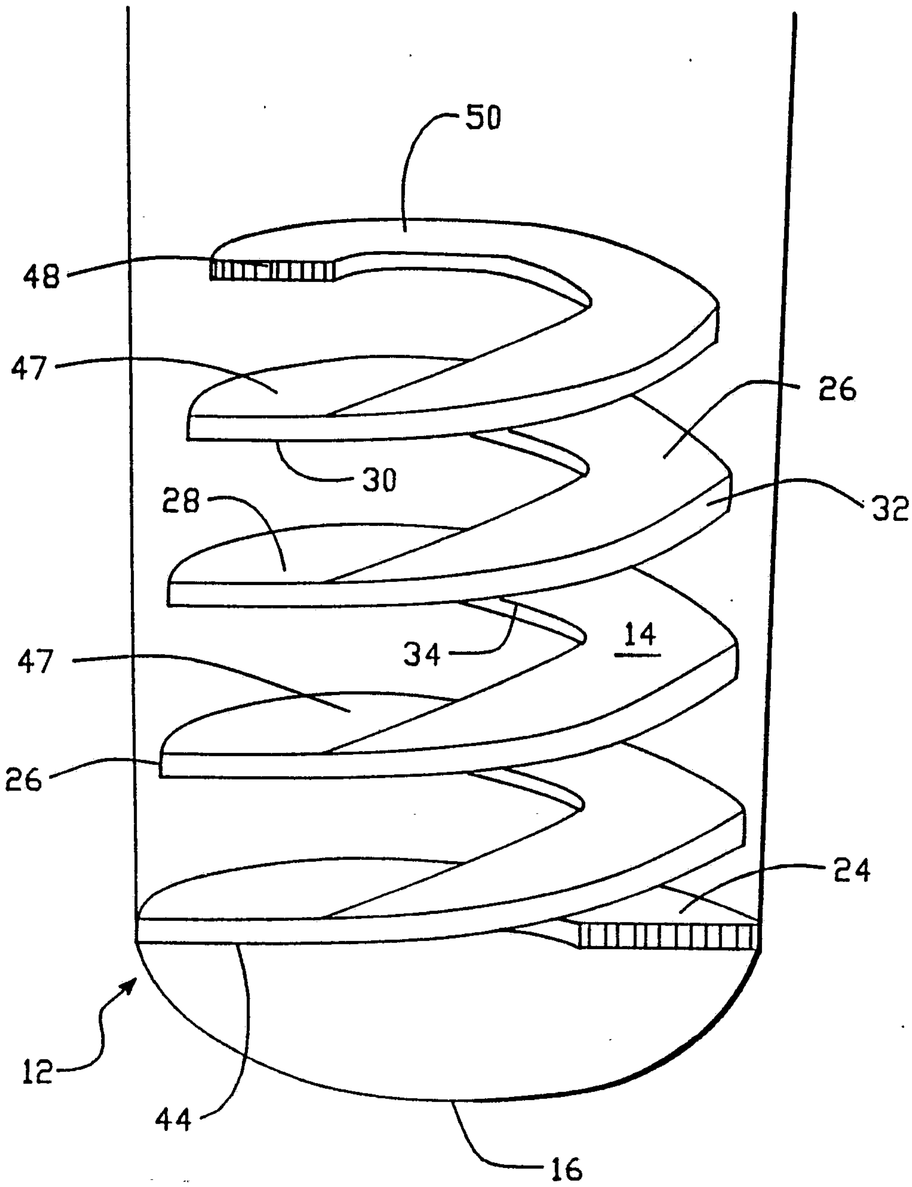

FIG.—4

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/06699

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): B01L 3/00
U.S. CL.: 422/102

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	422/56,58,61,99,101,102; 436/161,162,169,169,523,525,531,533,535

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

APS Above classes and spiral?

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X,P Y	US, A, 4,995,967 (van Driessche) 26 February 1991. see fig. 6.	1-2,4-9, 11-14 3,10
X Y	US, A, 3,768,978 (Grubb et al.) 30 Ocotber 1973, See Fig. 8.	1-2,4-9, 11-14 3,10
X Y	US, A, 4,551,435 (Liberti et al.) 05 November 1985. see entire document.	1-2,5,7-9 3,10

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
03 January 1992	22 JAN 1992
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Jan M. Ludlow ebw