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(NASA-CASE-MFS-28422-1) DROP DEPLOYMENT SYSTEM FOR CRYSTAL GROWTH APPARATUS Patent Application (NASA) 17 p C5CL 22A
Technical Abstract

DROP DEPLOYMENT SYSTEM FOR CRYSTAL GROWTH APPARATUS

This invention relates to a crystal growth apparatus utilizing a vapor diffusion method for growing protein crystals, and particularly to such an apparatus wherein a ball mixer (40, 104) is used to mix the fluids that form a drop (16) within which crystals are grown.

In the preferred embodiment, discrete vials (12 and 14), one for precipitate solution and one for protein solution, are each provided with a thin membrane (28) which is deformable to urge the contents of the vials through ball mixer (40) and then to drop support (64) in crystal growth chamber (16). This deformation is achieved by pressurizing a plurality of manifolds (41) also having a deformable membrane (42), which membrane bears against vial membrane (28). An opening (24) in an opposite end of the vials is closed by a puncturable septum (26), which is ruptured by a hollow needle (36) when manifolds (41) are pressurized, releasing the fluids to mixer (40). A tube (54) connecting mixers (40) and drop supports (64) is provided with a crystal storage region (70), which is capped at one end by a slide valve (74) and is sealed at an opposite end by crimping a narrowed portion (56) of tube (54).

In another embodiment of the invention, a shaft (84) set in a mounting block (94) is provided with transverse fluid deployment passageways (86), and recovery passageways (88) are each provided with free pistons (118). Crystal growth chambers (90) are positioned in block (94) along one side of shaft (84), and a plurality of fluid dispensing apparatus (102) each having a ball mixer (104) are disposed along an opposite side of shaft (84). In this embodiment, pressure applied to a region (108) behind free pistons (112) forces them forward, moving fluid contained in chambers (114) through ball mixers (104) and to drop supports (106) via transverse passageways (86). To recover the crystals, shaft (84) is rotated to align passageways (88) with drop supports (106) and suction applied behind pistons (118) to draw pistons (118) and the remainder of the drop and crystals therein into recovery regions (88). Shaft (84) is then rotated to an intermediate position to store the fluid and crystals.

Particular novelty of this invention lies in utilizing a ball mixer to completely mix the precipitate and protein solutions prior to forming the drop. Additional novelty lies in details of construction of the vials, the fluid deployment system, and the fluid storage system of the preferred embodiment. Still further, it is believed that details relating to construction of the
alternate embodiment are new.

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DATE FILED: December 18, 1990

SERIAL NO. 07/629,740
DROP DEPLOYMENT SYSTEM FOR CRYSTAL GROWTH APPARATUS

Origin of the Invention

The invention described herein was made by an employee of the United States Government and may be manufactured and used by or for the Government for governmental purposes without the payment of any royalties thereon or therefor.

Field of the Invention

This invention relates generally to protein crystal growth devices, and more particularly to such an apparatus for use in a microgravity environment wherein protein and precipitate solutions are stored in separate containers and mixed by a ball mixer upon deployment.

Background of the Invention

In the past, microgravity protein crystal growth experiments utilizing a well known vapor diffusion method for concentrating a drop of protein solution within which protein crystals are grown have typically taken place in an apparatus housing a number of crystal growth experiments which are activated simultaneously. In one particular embodiment of such apparatus, crystal growth chambers are each provided with a wicking element dampened with a precipitate solution of a concentration calculated to draw solvent from the drop at a selected rate over a predetermined period of time, slowly concentrating the protein solution and allowing highly ordered protein crystals to form. The protein droplet is situated at the end of a single pipette or side-by-side pipettes located near the wicking element, with the pipettes each coupled to a small syringe containing, in the single pipette version, a pre-mixed solution of precipitate and protein solution. When deployed, the pre-mixed
solution forms a drop at the end of the pipette. Each
pipette of the side-by-side version is coupled to a
separate syringe, one containing the protein solution
and the other containing a precipitate solution, with
mixing of the solutions occurring upon deployment.
This mixing may be achieved by repeatedly cycling the
two fluids into and out of the pipettes and syringes,
or by simply allowing the fluids to mix by diffusion
after the drop is deployed. The syringes are filled
prior to flight, and the pipettes capped to prevent
fluid loss by plugs connected to a common operating
mechanism. When the operating mechanism is operated,
all pipettes are uncapped simultaneously. Likewise,
plungers of the syringes are also coupled to a common
operating mechanism so that when operated, the drops
are deployed simultaneously.

While this type device has been proven to work
relatively well during several Space Shuttle flights,
problems have occurred. Most notable among these
problems is the difficulty of achieving complete
mixing, especially when dissimilar fluids are mixed.
Another problem is that the mechanism that deploys the
drops is not designed for repeated cycling, and
possibly may fail. Additionally, in some instances,
the drops were lost due to the relative instabilities
between the drops and pipettes during maneuvers in
space. Further, cycling of the fluids as described
introduces air bubbles therein, and the mechanical
action introduces small but unacceptable temperature
rises in the crystal growth enclosure, which is
temperature-regulated to ±0.1 degree C. Still
further, as the syringes are constructed of
dopolysulfone, a translucent material, the solutions are
difficult to load and evaluate, and the capping
procedure cannot be evaluated for its sealing and
compatibility before flight. Further yet, large
crystals may not be collectible, and marginally
collectible crystals may become damaged as they are
drawn back into the small openings of the pipettes, or otherwise become "hung" on a surface between the two pipettes.

Accordingly, it is an object of this invention to provide an improved crystal growth apparatus that more efficiently mixes precipitate and protein solutions, is better configured to support a drop of solution, and can capture and protect crystals of the largest size grown.

Summary of the Invention

A crystal growth apparatus is constructed wherein a vapor diffusion method is used to control crystal growth in a plurality of closed growth chambers each having a wicking element dampened with a precipitate solution, and a drop support proximate each wicking element for supporting a drop of fluid in which crystals are grown. Additionally, for each growth chamber, a pair of fluid containers are coupled to a mixer in turn coupled to the drop support, for mixing fluids in the containers, after which the mixed fluids are moved to form a drop on the drop supporter. After a predetermined period of time during which crystals are grown, remaining portions of the drops and crystals therein are drawn into separate storage regions, one for each drop, and sealed for later study.

Brief Description of the Drawings

Fig. 1 is a view, partially in section, of an embodiment of the present invention.

Fig. 2 is a sectional view taken along lines 2-2 of Fig. 1.

Fig. 3 is a cut-away view of a ball mixer of the present invention.

Fig. 4 is a sectional view taken along lines 4-4 of Fig. 3.

Fig. 5 is a view, partially in section, of an
alternate embodiment of the present invention.

Fig. 6 is a view illustrating particular details of the alternate embodiment shown in Fig. 5.

Description of the Preferred Embodiment

Referring to Fig. 1, a system 10 is shown for storing a precipitate solution and a protein solution in separate vials 12 and 14, and for deploying these solutions in a crystal growth cavity 16 having a wicking element 18 moistened with a precipitate solution.

Vials 12 and 14, one for precipitate solution and one for protein solution, are each constructed as shown in Fig. 1 having a hollow interior 20 for storage of one of the solutions, and a generally rounded end 22 provided with an opening 24 centrally located therein. A thin septum 26 is mounted in opening 24, and sealably contains the solutions in vials 12 and 14 prior to deployment. Sealing an opposite end 26 of each of vials 12 and 14, and in contact with the solution therein, is a thin, flexible diaphragm 28. The vials are slidably disposed in receptacles 30 each configured at an interior end 32 to match the rounded ends 22 of vials 12 and 14, with each interior end 32 provided with a hollow needle 36 aligned with opening 24 of vials 12 and 14. At an opposite end 38 of receptacles 30 is a hollow enclosure or manifold 41 provided with a flexible diaphragm 42 generally in contact with the respective vial 12 and 14 and its diaphragm 28. A source of hydraulic or pneumatic pressure 43 is in turn connected to each of manifolds 41 via conduit 45 such that when pressure is provided to manifolds 41, manifold diaphragm 42 applies pressure to the respective vial and its diaphragm 28, moving vials 12 and 14 in receptacles 30 and transferring pressure to interiors 20 of vials 12 and 14 to force the solutions upwardly into mixers 40. Needles 36 are each connected
via tubes 37 and 38 to a conventional ball mixer 40, such as the ball mixer disclosed in Biophysical Journal, Vol. 24, published in 1988, pages 2-20.

In this type mixer, as shown in Figs. 3 and 4, the first of the fluids to be mixed is directed through an axial, hollow inlet opening 42 to an asymmetrically-shaped ball 44 spaced from sides 46 of a mixer housing 48, and supported by means not shown. Ball 44 is configured having a plurality of opposed jets 50 positioned as shown and through which a portion of the first fluid is directed. The remaining portion of the first fluid flows between ball 44 and sides 40 adjacent ball 44. The second fluid is introduced into mixer 40 by a plurality of ports 52 positioned approximately 45 degrees with respect to jets 50, and slightly upstream ball 44. As the two fluids flow through mixer 40, the reduced-in-diameter flow path therethrough provides a venturi effect, increasing velocity of the fluid flow and creating turbulence that thoroughly mixes the fluids they as they flow around ball 44, the fluids exiting mixer 40 through exit tube 54.

Tube 54, constructed as shown in Fig. 1, is provided with a narrowed portion 56 constructed of a flexible silicone polymer material with a sheath 58 of thin stainless steel or like crimpable material encircling portion 56. A cam driven wedge 60 is movably positioned adjacent portion 56, with wedge 60 positioned to crimp sheath 58 when driven on a flat side 61 by cam 62, effecting a closure of tube 54 at narrowed portion 56. From narrowed portion 56, tube 54 continues to a drop support end 64 terminating in crystal growth chamber 16, drop support end 64 having a beveled inner region 66 which is better adapted to support a drop of fluid 68 than a flat-tipped pipette generally used in the prior art. The internal dimensions of this drop support end portion 64 is constructed to form a storage region 70 for grown
crystals, such storage region extending between narrowed portion 56 and drop support end 64 and having a diameter and length to accommodate the largest crystals grown in drop 68. Additionally, beveled inner region 66 provides a funneling effect to channel crystals into storage region 70, reducing the probability that crystals may become "hung" at the end of drop support 64.

Drop support ends 64 may be set flush in a rigid support 72 (Figs. 1 and 2), or as shown, ends 64 may protrude slightly above support 72. A slidable and liftable valve member 74 positioned over support 72 is operated by a cam member 76, with valve member 74 having openings 78 disposed to be selectively positioned in registry with support ends 64. Cam member 76 is provided with a frictional surface 77 which bears on an underside of valve member 74, allowing it to simultaneously lift and slide valve member 74 to cover drop support ends 64 or bring openings 78 into registry with drop support ends 64. Valve member 74 is biased against support 72 by a plurality of resilient sponge rubber springs 80, which are configured as shown in Fig. 1, with the wicks 18 also being resilient, the wicks positioned in a rectangular region between springs 80. Wicks 18 are each provided with a depression or cavity 82 adjacent drop support ends 64, depressions 82 forming the crystal growth chamber 16.

In operation, and referring to the embodiment of Fig. 1, the vials are separately loaded with precipitate and protein solutions and placed in their respective receptacles 30. After being situated in a microgravity environment, drops 68 are deployed by applying pressure to manifolds 41, distending diaphragms 42 and forcing vials 12 and 14 to "bottom out" in the contoured ends 32 of receptacles 30. When this occurs, needles 36 puncture septums 26, releasing the contents of each of vials 12 and 14 to be mixed by
mixers 40. Continued application of pressure causes manifold diaphragm 42 to bear upon and distend vial diaphragm 28, forcing fluids in the vials to be forced through mixers 40 and exit tubes 54 to drop support 64 where the drops are formed. At the end of a selected period of time wherein crystals are grown, the drops are recovered by removing all or some of the pressure in manifolds 41 and allowing diaphragms 42 to relax. This draws the drops and crystals into storage regions 70 of tubes 54, after which wedges 60 are operated on flat side 61 by cams 62 to crimp sheaths 58 and permanently close tubes 54 at narrowed portion 56. The slide valve 74 is then operated to close storage regions 70 at drop supports 64. To recover the crystals, the wicks and sponge springs are removed, exposing the slide valve, which is also removed, after which the storage regions 70 are removed with a special extraction tool (not shown) that cuts narrowed portion 56 at the mixer side of crimped sheath 58. At this point, the fluid and crystals may be removed or the storage portions 70 of tubes 54 may be capped and stored for later study.

An alternate embodiment of this invention is shown in Fig. 5. Here, a Teflon™ (or other inert material) shaft 84 is closely fitted in and rotatably mounted in a cylindrical channel 92 cut, machined, or molded in a rigid block of material 94, this material selected to be compatible and non-reactive with fluids used therein. Shaft 84 is provided with transverse fluid deployment passageways 86 and separate transverse storage and recovery passageways 88 each having a free piston 118 (Fig. 6), passageways 88 positioned normal to and offset from passageways 86. A plurality of crystal growth chambers 90, each having a drop support opening 106 as described above, are positioned in block 94 on one side of shaft 84, with a like plurality of fluid dispensing apparatus 102 positioned in block 94 on an opposite side of shaft.
Fluid dispensing apparatus are each constructed as shown in Fig. 5, with a free piston 112 disposed to move the fluid in containment regions 108 through mixers 104 responsive to pressure from bore 101. Storage and recovery passageways 88 are of a larger bore than deployment passageways 86, also as described above, to capture and store crystals of the largest size grown. A thumbwheel 96 connected to one end 98 of shaft 84 allows shaft 84 to be rotated, either manually or by electronic means (not shown), and the opposite end of shaft 84 is threaded and rotatably supported in a threaded portion 100 of channel 92 to rotate and move shaft 84 longitudinally, selectively aligning growth chambers 90 with fluid deployment passageways 86 or recovery and storage passageways 88. Additionally, pressure passageway 101 for communicating pressure to dispensing apparatus 102, when deployment passageways 86 are aligned with dispensing apparatus 102 and drop supports 106, is coupled to a source of pressure or suction 111 via bore 109 (solid lines) in shaft 84, while suction passageway 105 for communicating suction to recovery and storage passageways 88 is couplable to source 111 via a bore 107, shown in its actual position (solid lines) and its operative position (dashed lines). Source 111 of pressure or suction, for example, may be a manually operated syringe, for pneumatically generating the pressure or suction, or an automated, regulated source of pressure or suction. The ball mixers 104 are mounted proximate dispensers 102, but may be mounted in shaft 84 (not shown).

In this embodiment, the solutions to be mixed are contained in cylindrical or tubular containment regions 108. Regions 108 are coupled at one end 110 via conduits 113 and 115 with ball mixers 104, and as stated, are provided with free pistons 112 at the opposite end 114. A single opening 116 in end 114 of regions 108 is connectible to source of pressure 111.
which provides energy to move pistons 112 in order to deploy the fluids through mixer 104 to form the drop at drop support openings 106.

For recovering the remainder of the drop and crystals therein, the recovery and storage passageways 88 in shaft 84 are also provided with free pistons 118 (Fig. 6). Here, as one end 120 of the storage passageways 88 are aligned with the source of suction 111, the opposite ends of passageways 88 are also aligned with drop support openings 106. This allows the remaining portion of the drop and crystals therein to be drawn into storage passageways 88 responsive to suction applied to piston 118.

For the embodiment shown in Fig. 4, operation is as follows. The fluids are loaded into channels 108 with pistons 112 behind the fluid, and shaft 84 rotated to a position to seal dispensing apparatus 102, this position being other than a position of alignment of either deployment passageways 86 or recovery and storage passageways 88 with deployment apparatus 102 and drop support openings 106. Upon deployment, shaft 84 is rotated, bringing deployment passageways 86 into registry with deployment apparatus 102 at one end and drop support openings 106 at the other end. This action also aligns source 111 of pressure via passageways 101, 109, and 103 with dispensing apparatus 102, which pressure being sufficient to move pistons 112 in channels 108 to discharge the fluids through mixers 104 to form the drops at drop support openings 106 in crystal growth chambers 90. For recovering the remainder of the drops and the grown crystals after the requisite time period has elapsed, shaft 84 is again rotated, bringing storage regions 88 in shaft 84 into registry with growth chambers 90 at one end and with suction passageways 103, 107, and 105 at the opposite end. This suction is sufficient to draw piston 118 back in passageways 88, which in turn draws the remainder of
the drops into storage regions 88. The shaft is then rotated to the intermediate position, sealing storage regions 88. To remove the crystals, the shaft is removed and the crystals flushed from the respective storage regions and stored in separate vials, or capped and stored for later study.

Having thus described our invention and the manner of its use, it is apparent that incidental modifications may be made thereto that fairly fall within the scope of the following appended claims, wherein we claim:
Abstract of the Disclosure

This invention relates to a crystal growth apparatus (10) generally used for growing protein crystals wherein a vapor diffusion method is used for growing the crystals. In this apparatus, a precipitating solution and a solution containing dissolved crystalline material are stored in separate vials (12, 14), each having a resilient diaphragm (28) across one end and an opening (24) with a puncturable septum (26) thereacross at an opposite end. The vials are placed in receptacles (30) having a manifold (41) with a manifold diaphragm (42) in contact with the vial diaphragm at one end of the receptacle and a hollow needle (36) for puncturing the septum at the other end of the manifold. The needles of each vial communicate with a ball mixer (40) that mixes the precipitate and protein solutions and directs the mixed solution to a drop support (64) disposed in a crystal growth chamber (16), the drop support being a tube with an inner bevelled surface (65) that provides more support for the drop (68) than the tubes of the prior art. A sealable storage region (70) intermediate the drop support and mixer provides storage of the drop (68) and the grown crystals.