NASA CASE NO. MFS-28986-1

PRINT FIG. #2

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APPARATUS FOR DIFFUSION CONTROLLED DIALYSIS UNDER MICROGRAVITY CONDITIONS

The instant invention is directed to an apparatus for permitting mixing of solutions under microgravity conditions suitable for growing crystals by dialysis. The apparatus is primarily adapted for practicing the dialysis method of protein crystal growth taking advantage of long duration flight opportunities.

The apparatus 10 comprises a housing 12 within which a plurality of chambers 20, 22 are formed. A separate dedicated cylindrical valve 24 is positioned between the chambers of each pair of chambers. A plug 44 having an internal cavity 46 for containing a protein solution closes one of the chambers 20, and a dialysis membrane 46 separates the protein solution from the chamber when the plug is in place. Another plug 50 closes the other chamber of each pair. One of the chambers may contain a wick 56. The housing 12 may have portions 16, 18 of different lengths such that the chambers of one portion may be of a different length than the chambers of the other portion. Thus, the differences in crystal number and size relative to the rate of approach to critical supersaturation offered by different path lengths may be demonstrated and optimized. The wicking 56 permits diffusive mixing to control the critical approach to supersaturation via vapor pressure equilibrium. Precipitating solution and solvent may be placed in one or both of the chambers of a pair and at microgravity conditions, the valve 24 may be opened to permit diffusive mixing to commence.

The novelty of the invention appears to lie in the use of individual valves so that selected chambers may be activated sequentially at different times. The apparatus permits the controlled approach to supersaturation in the dialysis method or vapor pressure equilibrium. Additionally, the use of different chamber lengths in a unitary housing appears to be novel.

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PATENT APPLICATION

APPARATUS FOR DIFFUSION CONTROLLED DIALYSIS UNDER MICROGRAVITY CONDITIONS

ORIGIN OF THE INVENTION

BACKGROUND OF THE INVENTION

(1) FIELD OF THE INVENTION

This invention relates to apparatus for growing crystals under microgravity conditions, and more particularly to an apparatus for controlling the precipitant concentration in the dialysis method of protein crystal growth by diffusive mixing.

(2) DESCRIPTION OF THE PRIOR ART

One of the methods of growing protein crystals or other macromolecules is the dialysis method in which the protein or other molecular solution is physically isolated from the precipitating agent by a semi-permeable membrane. Upon activation, the precipitating agents diffuse through the membrane and mix with the protein solution. Another method of growing macromolecules is

the vapor diffusion, which decrease the solubility and concentrate the molecules to be crystallized by means of solvent evaporation.

Under gravity conditions disruptive convection currents significantly effect the crystal size and quality. Under microgravity conditions disruptive convection currents are avoided so that mixing is achieved by diffusion, thereby allowing a slower approach to critical supersaturation of the dissolved protein. This reduces the number of nucleation sites created and increases the size of the crystals which are grown since additional crystalline material may be deposited about the nucleii of the crystals formed. Thus, protein crystals may be grown in microgravity environments to produce crystals which are substantially larger than those grown under a gravity environment. The crystals are also of a higher quality and may be used in X-ray structure determination to permit more precise information to be obtained about the protein molecules. Additionally, during the actual crystal growth process (irrespective of the nature of approach to supersaturation), when a crystal grows in solution under unit gravity, the solution directly adjacent to the crystal surface becomes depleted in solute and the surrounding becomes less dense. This results in a growth plume, a convective disturbance called "solutal convection." In microgravity there is an absence of solutal convection and the crystal growth process becomes primarily diffusion limited which results in larger and more perfect crystals. Such information is of vital importance in advancing the development of new biotech pharmaceuticals, drugs and medicines.

In Carter et al U.S. Patent No. 4,909,933 there is proposed an apparatus for carrying out crystal growth

under microgravity conditions by the dialysis method. The apparatus includes a housing having a number of pairs of chambers, each pair of chambers having respective aligned openings separated by a cylindrical valve member common to the other pairs of chambers. The valve member may be rotated to simultaneously open or close communication between each pair of chambers of all of the pairs of chambers. This apparatus thus may be employed when a number of solutions are to be mixed at one time. Additionally, in accordance with the aforesaid patent, when carrying out dialysis for protein crystallization one of the chambers was filled with solution and sealed while dialysis buttons or membrane sacks had to be placed within the other chamber which was then sealed by a cap having a puncturable resealable elastic material. A second solution may be introduced into the chamber by a syringe needle which punctures the elastic material which thereafter reseals itself.

In proposed long duration space flights, a substantial number of experiments concerning protein crystallization are proposed for obtaining crystals of various proteins. It is thus desirable that crystallization by the dialysis method take advantage of diffusive mixing process to control the approach to supersaturation and that more control be provided over the dialysis or diffusive mixing processes performed during such microgravity environments so that the opportunities for growing such crystals may be custom tailored.

SUMMARY OF THE INVENTION

Consequently, it is a primary object of the present invention to provide an apparatus for growing protein crystals under controlled conditions in microgravity

environments.

It is another object of the present invention to provide an apparatus for growing protein crystals under microgravity conditions wherein protein crystals may be grown within a plurality of pairs of chambers, the growing process being initiated or activated sequentially at different intervals in each individual pair of chambers.

It is a further object of the present invention to provide apparatus for performing the dialysis method of protein crystallization under microgravity conditions wherein a protein solution may be contained within a cap having a dialysis membrane and which closes one of a pair of chambers, there being a plurality of pairs of chambers with the chambers of each pair separated by a dedicated valve member that may be selectively actuated to communicate the respective pair of chambers and permit one or more precipitating agents to diffuse through the membrane and mix with the protein solution.

Consequently, the present invention provides an apparatus for implementing crystal growth by allowing mixing of solutions under microgravity conditions, the apparatus comprising a housing having a plurality of pairs of chambers, the chambers of each pair being disposed for communication with each other through a valve which may open or close such communication. Each pair of chambers has a separate valve which is selectively controlled so that the individual pairs of chambers may be activated sequentially at selected intervals to take advantage of long duration flight opportunities. Protein solution, or other potential crystal growth solution, may be located within a small cavity in a cap which closes one of the chambers of a pair, the cavity in the cap

being closed by a dialysis membrane. Precursor solutions or precipitating agents may be placed in one or both of the chambers and the chambers sealed. When the apparatus is in a microgravity environment, each valve may be selectively rotated at an appropriate time. Optimum conditions for each dialysis experiment may be customized by varying the length of the chambers in selected pairs thereof. Conversely, valves of different orifice diameters may be utilized to control the diffusive mixing rate, thereby decreasing the overall length requirements of the device while achieving the same objectives. Additionally, wicking material may be placed in one or both of the chambers of a pair to control the diffusive mixing and thereby control the critical approach to supersaturation by vapor pressure equalization. The wicking may also be placed in or near the valve orifice for controlling the propagation of turbulent mixing produced when the valve is opened during activation. The apparatus may be utilized to perform liquid-liquid diffusion experiments in substantially any type of crystal growth application.

BRIEF DESCRIPTION OF THE DRAWINGS

The particular features and advantages of the invention as well as other objects will become apparent from the following description taken in connection with the accompanying drawings in which:

Fig. 1 is a fragmentary perspective view illustrating an apparatus incorporating the principles of the present invention and illustrating an embodiment depicting a series of chambers having two different lengths;

Fig. 2 is a cross sectional view taken substantially along line 2-2 of Fig. 1;

Fig. 3 is a fragmentary cross sectional view similar

to Fig. 2, but illustrating a wicking material disposed in one of the chambers;

Fig. 4 is an elevational view of one of the valves; and

Fig. 5 is an enlarged view of the protein solution containing cap illustrated in Fig. 2.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Referring now to the drawings, apparatus **10** constructed in accordance with the present invention for carrying out protein crystallization by dialysis and other chemical syntheses utilizing diffusive mixing, comprises a housing **12** which may be constructed from lightweight non-corrosive chemically inert materials, such as durable plastics or from stainless steel or other metal alloys. Although not illustrated, it may desirable to construct the housing out of a clear plastic material such as PLEXIGLASS (methyl acrylate plastic), polyethylene or TEFLON (polytetrafluorethylene) so that it may be possible to view the ongoing process visually. Otherwise, it may be desirable that a number of windows (not illustrated) above all or some of the chambers may be utilized to view and monitor the reactions. The housing **12** preferably has a substantially rectangular configuration, and may have stepped down portions such as illustrated at **14** such that at least two different length portions **16, 18** may be formed for reasons hereinafter made clear.

Formed in the housing **12** are a plurality of pairs of elongated cylindrical chambers **20, 22** extending through the housing, and since each pair of chambers is substantially identical, except for variations in the length of certain of the chambers, only one pair is illustrated in Fig. 2. Disposed between each pair of

chambers is a respective rotary valve member 24 comprising a cylindrical body member 26 with its axis of rotation extending transverse to the axes of the chambers and having an internal orifice or passageway 28 extending diametrically therethrough and adapted to be selectively aligned with the respective chambers 20, 22. The body of each valve member 24 includes a pair of O-rings 30, one above and one below the passageway 28, to provide a leak-free seal between the valve and the exterior of the housing. A lock ring in the form of a split ring or the like 32 secures the valve within the housing, while a disk-shaped cap 34 is disposed at one end of the valve body, i.e., the upper end, the exterior face of the cap being substantially planar with the upper surface 36 of the housing 12. A slot 38 or other indentation formed in the upper surface 36 of the cap provides a means for rotating the cap and thus the valve to selectively align the passageway 28 with the chambers 20, 22 to provide communication between the chambers as illustrated in Fig. 2 or to close such communication.

Each of the chambers 20, 22 open onto a respective enlarged threaded or tapped bore 40, 42 at the end of the chambers remote from the valve member 24. Threaded into the bore 40 to close the chamber 20 is a plug 44 having an internal cavity 46 adjacent the end of the plug which is received within the bore 40. A semi-permeable dialysis membrane 48 covers or closes the cavity 46 so that a protein solution may be placed within the cavity covered by the membrane 48 and be acted upon by one or more precipitating agents which diffuse through the membrane. A similar plug 50, albeit without the protein solution cavity and dialysis membrane, is threaded into the bore 42 of the chamber 22. Each of the plugs

44, 50 includes a respective O-ring 52, 54 to preclude leakage from the respective chamber. Preferably, at least the cap 50 may be formed from a durable elastic material which permits a needle syringe to pierce the cap to remove air from the chambers and may permit filling of one or more of the chambers with fluids while the cap is in place. The cap 50 may thus contain a TEFLON (polytetrafluorethylene) coated silicon rubber septum.

Since there is a separate valve for each pair of chambers, dialysis may be initiated in one pair of chambers independently of the other pairs of chambers. Thus, dialysis may be activated sequentially at different times to take advantage of long duration flight opportunities. A precipitating solution and solvent may be placed in the chambers 22 with each of the respective valves closed, and the various valves may be opened at specified times to permit the solution to enter the chamber 20 and diffuse through the membrane 48. If two separate solutions are to be used, one may be received into chamber 20 with the valve closed prior to closing the chamber by the plug 44. After the chamber is closed by the plug, the second solution may fill chamber 22 and it may be closed by the plug 50, or the first solution may fill chamber 22 when the valve is closed and the second solution may be injected into the chamber 22 after the valve is opened and the first solution enters the chamber 20. A substantial number of possible modes of operation are thus available to a researcher with apparatus constructed in accordance with the present invention. Moreover, rather than placing the protein within the cavity 46, the protein may be contained within a variety of accepted forms, e.g. within a dialysis button, syringe, on a glass slip or the like.

Also the plug 44 may contain protein without using a membrane 48.

Additionally, by constructing the apparatus with at least two portions, 16, 18, having different chamber lengths, the differences in crystal number and size relative to the rate of approach to critical supersaturation may be demonstrated by the different path lengths. A housing having three or more different chamber lengths in three different length portions would of course further demonstrate additional rates of approach.

Moreover, as illustrated in Fig. 3, a wick 56 comprising polysulphone, high molecular weight polyethylene, or similar porous wick material either entirely within chamber 20 or a portion thereof, may permit diffusive mixing to be used to control the critical approach to supersaturation by vapor pressure equilibrium, as, for example, in the standard "hanging drop" technique. The wicking provides the required surface area for the vapor equalization process to occur during the growth of the protein crystals. When placed in or near the valve orifice, the wicking may also be used to control the propagation of turbulent mixing produced when the valve is opened during activation. The wick may be of various shapes, including a donut or annulus shape.

Accordingly, apparatus constructed in accordance with the present invention provides a controlled approach to supersaturation in the dialysis method of crystallization. It permits individual pairs of chambers to be activated sequentially at different times to take advantage of long duration flight opportunities and provides a simple inexpensive approach to control supersaturation in microgravity environments. Optimum

conditions for each crystallization can be customized by varying the chamber lengths or the valve orifice diameters.

Numerous alterations of the structure herein disclosed will suggest themselves to those skilled in the art. However, it is to be understood that the present disclosure relates to the preferred embodiment of the invention which is for purposes of illustration only and not to be construed as a limitation of the invention. All such modifications which do not depart from the spirit of the invention are intended to be included within the scope of the appended claims.

APPARATUS FOR DIFFUSION CONTROLLED DIALYSIS UNDER MICROGRAVITY CONDITIONS

ABSTRACT OF THE DISCLOSURE

Apparatus for implementing crystal growth by allowing mixing of solutions under microgravity conditions includes a housing within which a number of pairs of chambers are formed. The chambers of each pair are aligned and a rotary valve is positioned between the chambers of each pair. When the valve is in a first position one chamber of each pair may communicate with the other chamber. A separate valve is provided for each pair of chambers so that each pair of chambers may be activated independently of the others and sequentially at selected intervals. Protein solution may be located within a small cavity in a cap which closes one of the chambers of a pair, and the cavity in the cap is closed by a dialysis membrane. The length of certain pairs of chambers may differ from the length of other pairs of chambers to optimize conditions for various dialysis productions, and wicking material may be incorporated into selected chambers for controlling the critical approach to supersaturation.

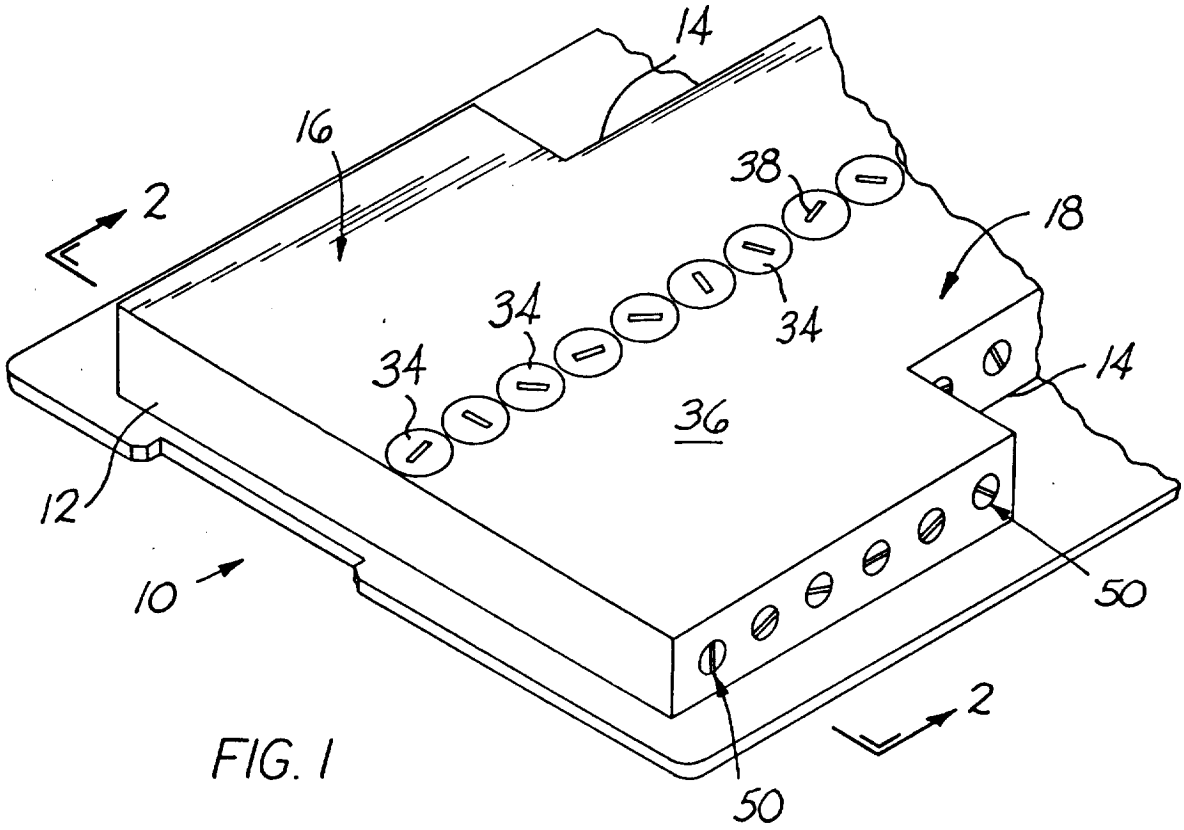

FIG. 1

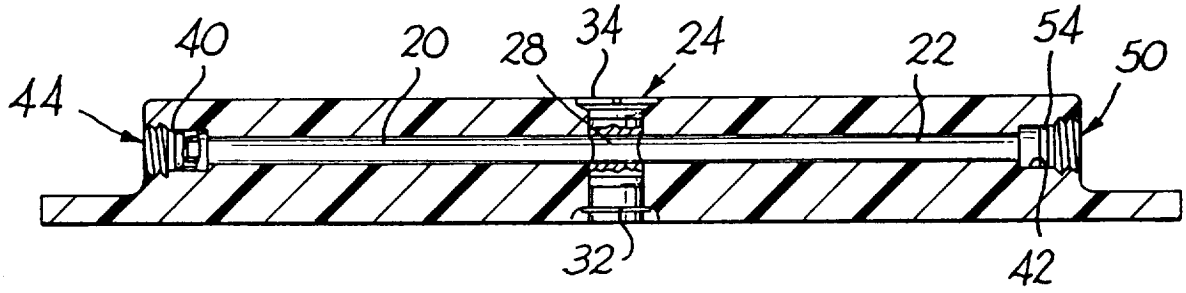

FIG. 2

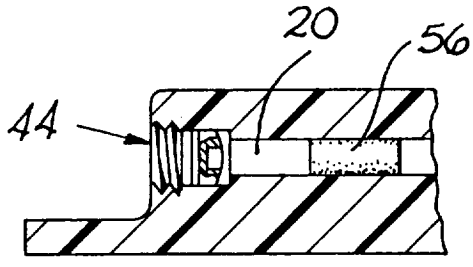

FIG. 3

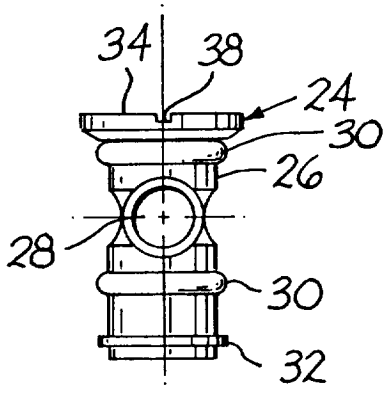

FIG. 4

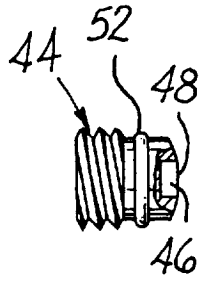

FIG. 5