The invention relates to a device for molecular and macromolecular crystallization. More particularly, the device comprises a well and a transparent cap for growing diffraction-quality protein crystals by conventional vapor diffusion techniques. The present device is particularly advantageous in that it allows the pre-filling of the well with a solution for transport and handling.
CRYSTALLIZATION METHODS FOR LABORATORY CAP AND WELL

FIELD OF THE INVENTION

[0001] The present invention relates to a device for handling molecular and macromolecular crystallization. More particularly, the device comprises a well and a cap assembly for growing protein crystals by conventional vapor diffusion techniques. The present device is particularly advantageous in that it facilitates the pre-filling of the well with a solution for transport and handling prior to utilization by a technician.

BACKGROUND OF THE INVENTION

[0002] Crystallography is an extremely useful tool for scientists, and is therefore a field of research attracting a lot of interest. It is a powerful means that provides precise and detailed description of the three-dimensional structure of the molecules, and is of great help in the understanding of their functions. Crystallography of macromolecules like proteins is extensively used today, academically as well as industrially.

[0003] Although three-dimensional structures of simple proteins have been obtained through crystallization methods, it is not always easy to obtain crystals from macromolecules. For example, the preferred conditions for the crystallization of a given molecule can take several hundred if not thousands of trials. As a result, means and methods have been developed to perform a great number of trials relatively quickly, including hanging-drop and sitting-drop methods. All such methods use the benefit of vapor diffusion to obtain the crystals.

[0004] The hanging-drop method is currently the most commonly used technique for scanning various crystallization conditions of macromolecules, such as proteins. It comprises suspending a droplet of approximately 2 to 20 µL of solution containing the macromolecule to be crystallized and a precipitating agent, over a precipitating solution, such as conventional polyethylene glycol 20% or ammonium sulfate 40%, contained in a reservoir or well. The system is then sealed hermetically. After a while, vapor diffusion of the solvent or solvent mixtures between the droplet and the solution in the reservoir reaches equilibrium. The end result is a decrease of water in the droplet, and an increase of the macromolecule and precipitating agent concentration therein, thus causing crystallization of the macromolecule in optimized conditions. The actual technique for the set up of the hanging-drop or sitting-drop experiments is a long and arduous work and has to be performed by qualified and skilled technical personnel.

[0005] Conventionally, a commercially available tray made of an inert thermoplastic material comprising a plurality of reservoirs or wells is prepared, and the precipitating solution is placed in each reservoir or well manually. The macromolecule solution is then mixed with a precipitating agent on a glass plate (coverglass) and the whole is inverted over the wells, thus making the macromolecule solution overhanging the well. Prior to placing a glass plate over a well, the rim of each well is greased to ensure a proper seal. Care must be taken when placing the plate over each well, since the grease can easily contaminate the macromolecule solution. The crystallization process is followed with the help of a microscope. After the crystal is obtained, the glass plate is removed. Again, this must be done with great care to prevent contamination of the crystallized macromolecule with grease, and/or breaking of the glass plate. On top of that, the plates are hardly reusable for any experiment because the grease is hard to remove, and some of it remains on the plates.

[0006] An advantage of the hanging-drop and sitting-drop methods is that they provide screening conditions for crystallization, and truly represent a microcrystallization technique. The vapor diffusion in the hanging or sitting drop allows screening of a large range of conditions and necessitates a relatively small amount of macromolecules. Further, it allows a relatively clear visualization of the results, and the eventual crystals are free, i.e., they do not adhere or are stuck to any surface.

[0007] Typically, several hundreds of experiments are required to find appropriate crystallizing conditions for the production of high quality crystals. Accordingly, hanging-drop and sitting-drop experiments are a very labor-intensive process demanding skilled and experienced technical personnel. For example, multiple aspirating and dispensing is steps of components, multiple greasing steps etc. must be performed in the experimental setup. Further, for each well, a separate coverglass must be manually inverted. The number and complexity of steps can therefore produce an undesirable wide variation in experimental results.

[0008] As stated above, grease is conventionally used to provide a seal between the well and the coverglass. Other ways for sealing the system have been proposed. For example, grease can be replaced with immersion oil or an adhesive tape. As with grease, these sealing means have serious drawbacks. Grease is not always easy to dispense around the upper rim of the well, and is a time consuming operation. Technicians repeating the operation thousands of times occasionally suffer physical pain to their hands. Other significant problems and risks are present when manipulating the crystal on a greasy cover slide. The cover slide sometimes breaks during the process, which may cause injury to the technician, in addition to losing the crystals. The immersion oil is also problematic. One has to use a determined volume of oil. Too much oil leads to contamination within the well, while not enough will lead to non-hermetic seal that may result in the evaporation of the precipitating solution. An adhesive tape allows quicker and simpler manipulations, but all experiments are sealed at the end of the set-up, thus introducing experimental variations between the 1st and the 24th drop. Further, crystals often stick to the tape, rendering impossible the recovery of the crystals, and the operations for the recovery of the drop are also problematic.

[0009] These conditions promoted the robotization of the procedure. Some automated crystallization devices already exist. The well-known Cyberlab-200™ apparatus dispenses solutions in wells, greases the upper rim of each well, pours droplet on cover is slides held by a vacuum arm, and places the cover slides over the wells. However, such apparatus still has some drawbacks, namely a complicated experimental set-up, and the notable use of grease. Further such apparatus is extremely expensive.

Because of its simplicity, the operations of filling the well with the precipitating solution, placing a drop of the macromolecule solution onto the cap and sealing the well by putting the cap in position over the well can be accomplished by any competent technician, and not only skilled personnel.

[0020] In a preferred embodiment of the invention, a plurality of wells are molded together, for example in a tray comprising 4 rows of 6 wells each, with corresponding transparent caps are provided thereon. The resulting tray and caps may also be optionally treated with a hydrophobic agent such as a silicogenic agent.

[0021] Because of the transparency of the cap and of the bottom surface of the well, crystallization can be followed with minimal handling, and without disturbing the vapor equilibrium within each well. Further, visualization of the results under the microscope are simple because the cap is made of a transparent or translucent (clear) material.

[0022] Preferably, the material of the tray and the cap are the same, and comprise materials that can be molded easily at a reasonable cost. The material should be stable for extended periods of time towards the various chemical products present in the well and onto the cap. The material should also preferably not absorb water, and be in good optical quality to facilitate work and observation under a microscope. Example suitable materials include various thermoplastics such as polystyrene, polypropylene, polycarbonate, polycrylate, polymethacrylate, acrylonitrile-styrene copolymers, nitrile-acrylonitrile-styrene copolymers, polyphenyleneoxide, phenox resin, etc., the most preferred material being polystyrene.

[0023] It is another object of the present invention to provide a crystal-forming device that allows the manipulation of the growth crystals under the microscope without any transfer from the cap, where solutions can be added directly without any transfer of the crystals, in a greaseless environment.

[0024] Another major advantage of the device of the present invention is that once a series of experiments is completed, the tray is readily reusable, simply by taking another series of caps containing a drop of a solution containing a macromolecule to be crystallized, and reinstalling the caps over the wells. Further, a given cap may be removed from its original well and locked onto another one containing a different precipitating solution.

[0025] The invention is also concerned with a method for forming crystals of a macromolecule, the method comprising the steps of dispensing a precipitating solution in a well; forming a droplet in a cap comprising locating members to lock the cap over the well; and locking and sealing the well. In a preferred embodiment, a ring made of an elastomeric material like polypropylene, an ethylene-propylene copolymer, Teflon™ etc., is preferably provided between the cap and the well. In a further preferred embodiment, the well can be filled in advance and tightly sealed, so that they a tray is provided to a technician in a “ready-to-use” manner.

[0026] Because of the ergonomics of the present invention, the cap is engaged easily so that there is no need for special manual dexterity comparatively to the use of conventional thin, fragile, microscope coverglasses. The presence of a cavity in the surface of the cap facing the bottom surface of the well allows the addition of liquid directly over the drop, after placing the cap upside down on a table,
without the need to transfer the crystals to another well, thus
limiting the manipulations that might ruin the fragile crys-
tals.

[0027] The use of the cap and well assembly of the present
invention can be automated in a straightforward manner by
providing the extremity of an automated arm with a simple
grip element having an end provided with a structure
adapted to releasably grip the cap. There is no need for
the application of grease or the manipulation of fragile cov-
glass pieces. The grip element may also be manipulated
manually by a technician, as described hereinbelow.

[0028] The cap and well assembly of the present invention
also finds applications in the field of cell cultures, molecular
or cellular biology etc.

[0029] In a most preferred embodiment of the invention, the
well is filled beforehand and sealed with the cap. The
technician therefore receives a “ready-to-use” assembly, thus
eliminating the time-consuming operation of filling each well
with the appropriate precipitating solution. The
buyer may therefore order as many assemblies as desired
with the same or different precipitating solutions. For ship-
ping purposes, the cap may be replaced on the assembly with
a film to prevent contact of the precipitating solution with
the cap. Such contact would necessitate the cleaning of the
cap prior to its use. One may also use a cap for shipping
purposes, and a different cap to carry out the experiments. It
is important that the well be sealed to avoid evaporation
and spilling of the precipitating solution, either during ship-
ment of the pre-filled wells, or during the experiments.

[0030] Referring to the drawings which illustrate preferred
embodiments of the invention, FIG. 1 illustrates a cap and
well assembly 10 which comprises a tray or base plate 12
provided with a plurality of wells 14 and corresponding caps
16. Assembly 10 may also include a cover 18 used for
shipping or storage purposes. The preferred form of cover 18
comprises with an insertion (not shown) at each corner that
allows retention of the cover over the caps without touching
them. Cover 18 further allows the storage of several trays of
experiments one on top of the other. Tray 12 comprises a rim
20 extending about the four side walls thereof, and is
provided with finger grip surfaces 22 such as those described
in U.S. Pat. No. 4,038,149, on two opposed side walls for
easier handling of the tray by the technician. Finger grip
surfaces 22 are provided to avoid mishaps, and greatly
facilitate handling of covered and uncovered trays. Cover 18
comprises a section 24 adapted to engage around finger grip
surfaces 22 for proper fitting over assembly 10.

[0031] FIG. 2 illustrates a section view of cap 16. As it can
be seen, cap 16 comprises a cylindrical slot 26 into which is
inserted an O-ring element 28 made of a resilient material.
Such material, although optional, is provided to ensure an
appropriate seal when cap 16 is fitted over upper rim 30 of
well 14. The inner surface 32 of slot 26 has a portion or ridge
34 extending planar surface 36 thereby forming a cavity 38.
Surface 36 may be concave or convex, but the planar
configuration illustrated on FIG. 2 is much preferred.
As stated above, the material of cap 16 is such that it is
sufficiently transparent or translucent so that cap 16 can be
placed directly under a microscope for observation and/or
manipulation of the crystals.

[0032] Each cap 16 comprises a pair of locking element 40
diametrically opposed to each other and comprising a ridge
portion 41. Cap 16 also comprises a further rim 46 provided
with a series of spacer 45 underneath. Once the precipitating
solution is poured into well 14, the technician puts cap 16
upside down on a flat surface and places a drop of the
macromolecule-containing solution onto surface 36. Cap 16
is then flipped over cautiously, and each locking element 40
is inserted into a corresponding opening 42 provided onto
the upper surface 44 of tray 12 until the abutment of upper
rim 30 of well 14 with the O-ring element 28 inside slot 26
is achieved. Cap 16 is then rotated so that locking elements
40 slide each into a slot 43 having a width smaller than that
of opening 42 and extending on a portion of the periphery
of well 14 until the upper surface of portion 41 is entirely under
upper surface 44, thereby efficiently sealing well and main-
taining cap 16 in place. In a most preferred embodiment, a
section 47 of portion 41 is tapered to facilitate sliding under
upper surface 44. To ensure even better locking and main-
tenance of the cap in position, a small bump (not shown) is
provided onto section 49 that is adapted to fit into a
respective recess (not shown) present under surface 44
after complete insertion of portion 41 under surface 44.

[0033] To put cap 16 in place onto well 14, or for removal
therefrom, a tool 48 may be used, as illustrated in FIGS. 3
and 4. Tool 48 comprises a body 50 divided in a portion 52
shaped in a manner such as to facilitate holding by the
technician or an automated arm; a cylindrical portion 54
with an external surface 57 having a circumference slightly
bigger than that of rim 46, and an internal surface 59 having
a circumference slightly smaller than that of rim 46. Tool
48 further comprises two diametrically opposed cap gripping
elements 60 each provided with a gripping finger 62. The
gripping element 60 can be provided onto internal surface
59, directly on rim 66, or onto external surface 57. In
operation, tool 48 is placed over cap 16 so that each finger
62 is inserted into a slot 64 cut into rim 46 until at least a
portion 65 of each element 60 is abutted onto rim 46. Tool
48 is then rotated until gripping fingers 62 are completely
engaged under rim 46, and the rotation is maintained until
the locking members 40 are aligned with slots 42. Cap 16 is
then simply pulled up. To reintroduce the cap in position, the
procedure is carried out in an opposite manner. The external
surface 53 of portion 52 should be planar, so that it can be
laid on a table or under a microscope in a stable manner, and
allow the technician to observe and/or work on the crystals.
To be able to work under a microscope directly, surface 53
must comprises an opening preferably corresponding to the
internal diameter of cylindrical portion 54 (see FIG. 4).

[0034] FIG. 5 illustrates another embodiment of the
present invention. The cap and well assembly 100 which
comprises a tray or base plate (not shown) provided with a
plurality of wells 112 and corresponding cap 114, which
comprises a cylindrical slot 116 into which is inserted an
O-ring element 118 made of a resilient material. As for the
previous embodiment illustrated, the O-ring, although
optional, is provided to ensure an appropriate seal.

[0035] Each cap 114 comprises a pair of locking element 120
diametrically opposed to each other and comprising a ridge
portion 122. Cap 114 is locked into position on the tray
by inserting each locking element 120 into a corresponding
opening 124 provided onto the upper surface 126 of the
tray until the lower surface 128 of cap 114 lies flat onto the
upper surface 126. Cap 114 is then rotated so that locking
elements 120 slide into a slot 130 having a width smaller
than that of opening 124 and extending on a portion of the periphery of well 112 until the upper surface of ridge portion 122 is entirely under upper surface 126, thereby efficiently sealing well and maintaining cap 114 in place.

[0036] FIG. 6 illustrates another simple variation of the present invention, wherein the upper surface 152 of a well 150 comprises a slot 154 along its circumference and adapted to receive an O-ring element 156 coupled to a cap 158. The section of slot 154 is such that is slightly smaller than that of element 156, so that upon insertion into the slot, a tight seal is formed by the locking of cap 158 to well 150 without the need of any adhesive or grease.

[0037] FIG. 7 illustrates yet another embodiment of the invention, wherein the cap 170 is screwed on the well 172.

[0038] The present cap and well assembly is particularly suitable for both hanging-drop or sitting-drop crystallization methods. With respect to the sitting-drop method, although not specifically illustrated in the drawings, anyone skilled in the art will readily appreciate that any conventional drop support can be inserted or molded into the well. Examples of such sitting-drop support include the Micro-Bridges™ or the glass sitting drop rods manufactured and sold by Hampton Research (Laguna Hills, Calif.).

[0039] Each well is carefully filled with a selected equilibrating solution. Subsequently, a selected protein drop is deposited on the cap. The shape and the texture of the lower surface can be varied to obtain optimum results for a particular protein solution being crystallized, for example, when lower surface tension solutions, including protein solutions containing detergents, are used. The addition of the equilibrating solution and the protein drops to the device may be carried out either manually or through commercial automated pipetting apparatus, and the sealing of the cap over the solution may also be carried out manually or in an automated manner.

[0040] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses or adaptations of the invention following, in general, the principles of the invention and including such departures from the present description as come within known or customary practice within the art to which the invention pertains, and as may be applied to the essential features hereinafter set forth, and as follows in the scope of the appended claims.

1. A method for forming molecular or macromolecular crystals comprising the steps of:
   dispensing a precipitating solution in a well having an open upper end;
   providing a droplet of a solution containing a molecule or macromolecule on a crystallization support formed on an underside of a cap;
   inverting the cap so the support is positioned over the open upper end of the well and the droplet is suspended over the precipitating solution contained in the well;
   engaging a first locking means associated with the cap and a second locking means associated with the well to simultaneously interlock the cap onto the well and to close and seal the open upper end of the well.

2. The method of claim 1 wherein the support is transparent, the well has a light transmitting bottom and a resilient member is disposed between each cap and each well to ensure the sealing thereof.

3. The method of claim 2 further including the step of observing the activities within the sealed well through the transparent support with the aid of light passing through the light transmitting bottom of the well.

4. The method of claim 1 wherein the cap and the open upper end of the well are tubular and the step of engaging includes rotating the first and second locking means relative to one another.

5. A method of claim 1 further comprising the step of pre-filling the well with the precipitating solution and sealing the well before shipment to a purchaser.

6. A method of forming crystals by vapor diffusion comprising the steps of:
   providing a tray having a base with a plurality of wells supported on the base with each well having an open upper end and a light transmitting bottom;
   providing a plurality of caps, each with a transparent crystallization support formed on an underside thereof;
   placing a precipitating solution within each well;
   placing a droplet of solution containing a molecule or macromolecule to be crystallized and a precipitating agent on each of the crystallization supports;
   inverting each cap so each crystallization support is positioned over each open upper end of each well and the molecule or macromolecule is suspended over the precipitating solution contained in each well; and,
   engaging a first locking means associated with each cap and a second locking means associated with each well to simultaneously interlock each cap onto a well and to close and seal the open upper ends of the wells.

7. The method of claim 6 further including the step of positioning a resilient sealing member on each cap so as to enhance the seal between each cap and each well without the need or use of a contaminating sealing material between each cap and well.

8. A method of claim 6 further including the step of examining and monitoring crystal growth under a microscope through the crystallization support with the aid of light passing through the light transmitting bottom of the well.

9. The method of claim 6, wherein the caps and the upper end of each well are tubular and the step of engaging includes rotating the first and second locking means relative to one another.

10. A method according to claim 6, further comprising the step of pre-filling each well with the precipitating solution and sealing each well before shipment to a purchaser.

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