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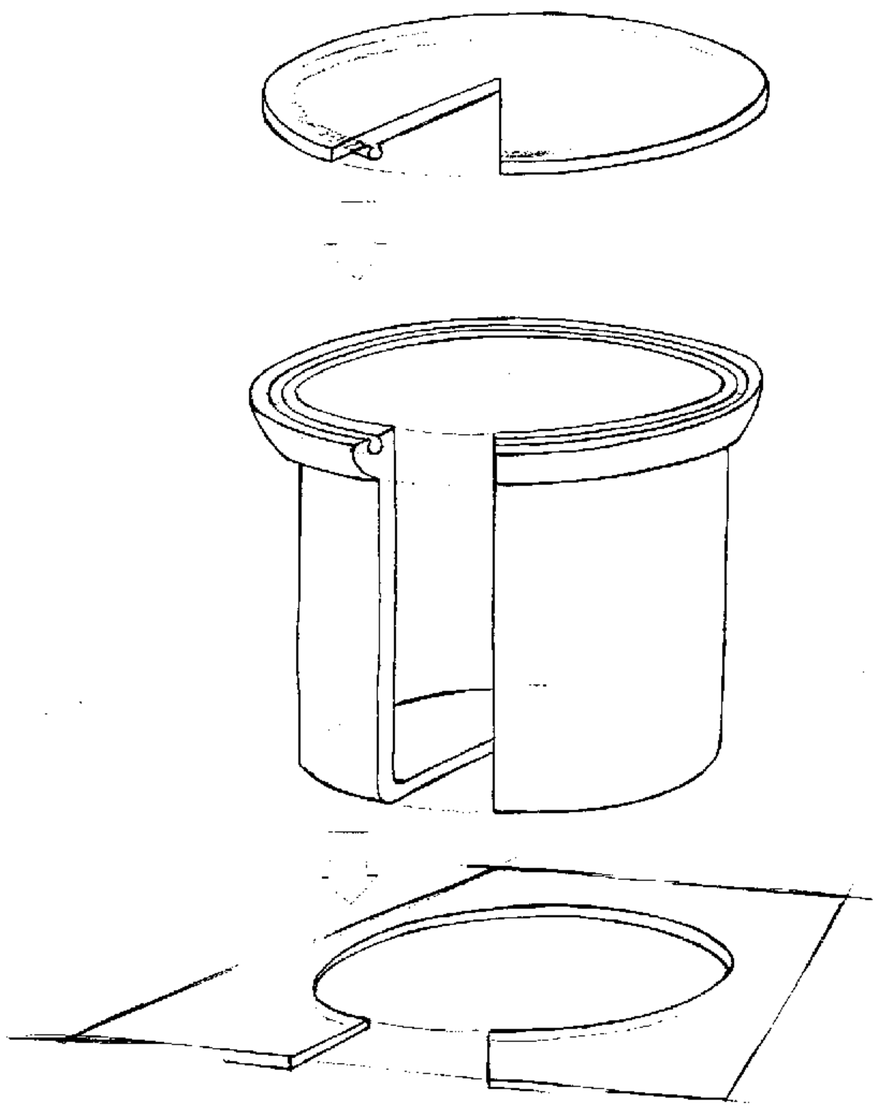
(54) **BOUCHON ET PUITS DE LABORATOIRE POUR DES METHODES DE CRISTALLISATION PAR GOUTTE SUSPENDUE**

(54) **LABORATORY CAP AND WELL FOR HANGING-DROP CRYSTALLIZATION METHODS**



(57) 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.

ABSTRACT OF THE DISCLOSURE

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.

TITLE

Laboratory cap and well for hanging-drop crystallization methods

FIELD OF THE INVENTION

The present invention relates to a device for handling molecular and macromolecular crystallization. More particularly, the device comprises a well and a cap 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.

BACKGROUND OF THE INVENTION

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.

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 hundreds 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. They all use the benefit of vapor diffusion to obtain the crystals.

The hanging-drop method is currently the mostly used technique. It comprises suspending a droplet (2-20 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, and this system is sealed hermetically. After a while, water vapor diffusion between the droplet and the solution in the reservoir reaches an equilibrium. The end result is a decrease of the concentration of water in the droplet water, and an increase of the protein and precipitating agent concentration therein, thus causing crystallization of the protein. The actual technique for the set up of the hanging-drop experiments is a long and arduous work and has to be performed by qualified technical personnel.

Conventionally, a 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. Such trays are readily available commercially. The protein solution is then mixed with a precipitating agent on a glass plate and the whole is inverted over the wells, thus making the protein overhanging the well. Prior to placing the glass plate over the wells, the rim of each well is greased to ensure a proper seal. Care must be taken when placing the plate over the wells, 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 very carefully to prevent contamination of the crystallized macromolecule with grease, and breaking of the glass plate. On top of that, the plates are not easily reusable for any experiment.

An advantage of the hanging-drop method is that it provides screening conditions for crystallization, and truly represents a microcrystallization technique. The vapor diffusion in the hanging drop allows screening of a large range of conditions and necessitates a relatively small amount of macromolecules.

Typically, several thousands of experiments are required to find appropriate crystallizing conditions producing high quality crystals. Accordingly, hanging-drop experiments are a very labor-intensive process demanding skilled and experienced personnel. For example, multiple aspirating and dispensing steps of components, as well as multiple greasing steps and the like must be performed in the experimental set up. Further, for each well, a separate cover slip must be manually inverted. The number and complexity of steps can therefore produce a wide variation in experimental results.

As stated above, grease is conventionally used to provide a seal between the well and the covering plate. 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 are present when removing the crystal from a greasy cover slide. The cover slide sometimes breaks during the process, which may cause injury to the technician, in addition to loosing 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. 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

These conditions promoted the robotisation of the procedure. Some automated crystallization devices already exist. The well-known apparatus Cyberlab-200™ dispenses solutions in wells, greases the upper rim of each well, pours droplets on cover slides held by a vacuum arm, and places the cover slides over the wells.

It would therefore be highly desirable to develop a hanging-drop device for crystallizing macromolecules that would overcome the above drawbacks. Such device would eliminate the requirement of external means like grease, oil or an adhesive tape to seal the well and the cover, and would preferably be easy to manipulate. Ultimately, the process of experimental set up of the hanging-drop device would be greatly facilitated and accelerated, while simultaneously eliminating possible risks of contamination of the eventual crystals.

IN THE DRAWINGS

Figures 1 to 6 illustrate preferred embodiments of a device according to the present invention.

DETAILED DESCRIPTION OF THE INVENTION

It is an object of the present invention to provide a crystal forming device using vapor diffusion method. The device comprises a well and a transparent cap adapted to close the well and form a sealed volume, the well being sealed without the need to add a sealing material like grease, oil, adhesive tape and the like between the well and the cap. The cap is made of transparent material to allow examination and monitoring of crystal growth. The present device therefore represents an important advance in methods for growing crystals of macromolecules.

In a preferred embodiment of the invention, a tray comprising a plurality of wells with corresponding transparent caps are provided, so that several crystallization experiments can be carried out simultaneously. Such a tray can be used manually or through an automated system.

Because of the transparency of the cap, 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 formed with a clear material that preferably does not absorb water and is of good optical quality.

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 we can add directly solutions in a greaseless environment.

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 macromolecule to be precipitated, and reinstalling the caps over the wells.

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 engaging means to engage with receiving means in the well; and closing and sealing the well by engaging the engaging means of the cap with the receiving means of the well. In a preferred embodiment, a ring made of an elastomeric material like an organopolysiloxane, is provided between the cap lower surface and each well ridge. In a further preferred embodiment, the well can be filled in advance and tightly sealed, so that they can be provided to a technician in a ready-to-use manner.

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 cover slides. The formation of a depression on the cover slide versus the bend of the cap allows the addition of liquid directly over the drop without transferring the crystals to another well, thus limiting the manipulations that might ruin the fragile crystals.

Referring to the drawings, there are provided various embodiments of the device of the present invention for growing macromolecular or molecular crystals. Each

device comprises a well and a transparent cap adapted to be engaged with the well and form a seal. Because of the nature of the present invention, the wells are generally gathered in the form of a tray comprising a plurality thereof, each tray containing 24 wells. The tray is preferably molded in any suitable transparent thermoplastic material comprising a plurality of wells that are open at the top. The various engaging means between the cap and the well allow the sealing of the well without the need for grease, oil, tape or any other foreign sealing material. In a most preferred embodiment, the cap is provided with means allowing a technician to easily engage and disengage the cap. Also in a most preferred embodiment, a strip of an elastomeric material, i.e., an O-ring, is provided between well upper rim and the lower surface of the cap. Preferably, the lower surface of the well is planar, and made of transparent material of good optical quality, such as for example, thermoplastic materials like polycarbonate or polystyrene.

In a further preferred embodiment, the tray is of rectangular shape, and the 24 wells and the caps are cylindrical, and arranged in rows. Also, the tray is formed by an injection molding process from a clear moldable plastic, for example polystyrene or polycarbonate.

Preferably, the cap, is also formed by an injection molding process in the same manner and with the same material as for the tray, and is optionally treated with silicone.

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, and the sealing of the cap over the solution, can be done either manually or through commercial automated pipetting apparatus.

Once the experiment is completed, the user can cover the tray and the caps with a cover. Preferably, this cover as a planar, wall and a skirt. The preferred form of the cover is rectangular with insertion in corners that allows retention of the cover over the caps without touching them. This cover fits with the bottom ridge of the tray and allows the storage of several trays of experiments one on top of the other. Preferably, the cover is also made in the same manner and with the same material as the tray and the caps.

In order to avoid mishaps and greatly facilitate handling of the covered and uncovered trays, the tray is provided on opposite sides of its circumferential wall with finger grip surfaces which, with or without the cover on the tray, are accessible to a user's finger grasp for handling, including lifting the tray in an entirely convenient manner.

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 hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A device for growing molecular and macromolecular crystals comprising a well for receiving a precipitating solution, the well comprising receiving means for receiving corresponding engaging means from a cap to seal the well.

2. A device according to claim 1 wherein a strip of an elastomeric is inserted between the well and the cap.

3. A tray comprising a plurality of wells according to claim 1.

4. A device according to claim 3, wherein the tray is covered by a cover top to protect the tray from dust, temperature variation and allow a secure grasp of the covered tray by an individual.

5. A device according to claim 2, wherein the elastomeric material is an organopolysiloxane elastomer.

6. A device according to claim 1, wherein the engaging means and the receiving means are threaded.

7. A device according to claim 1 wherein the cap is transparent.

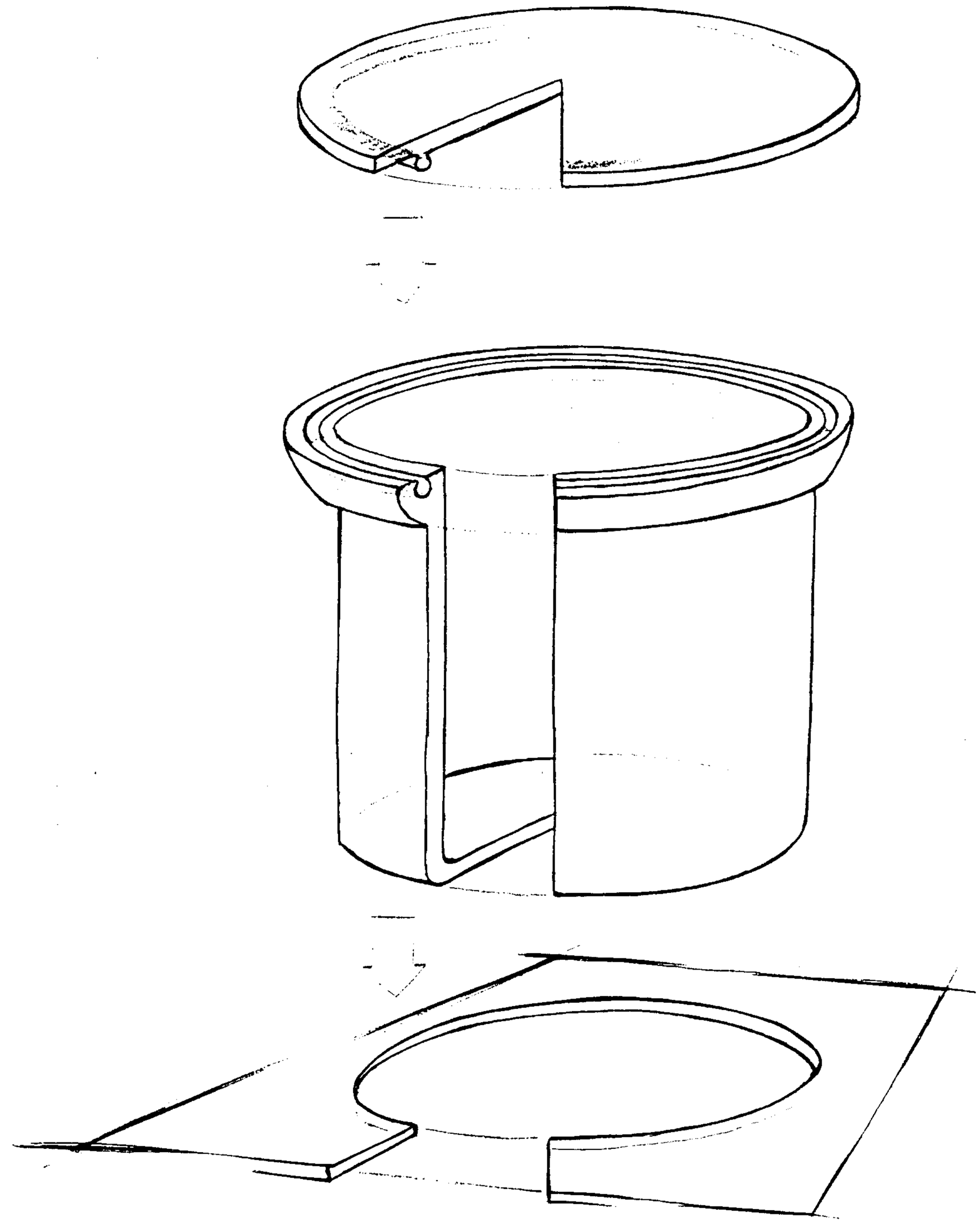


Fig. 1

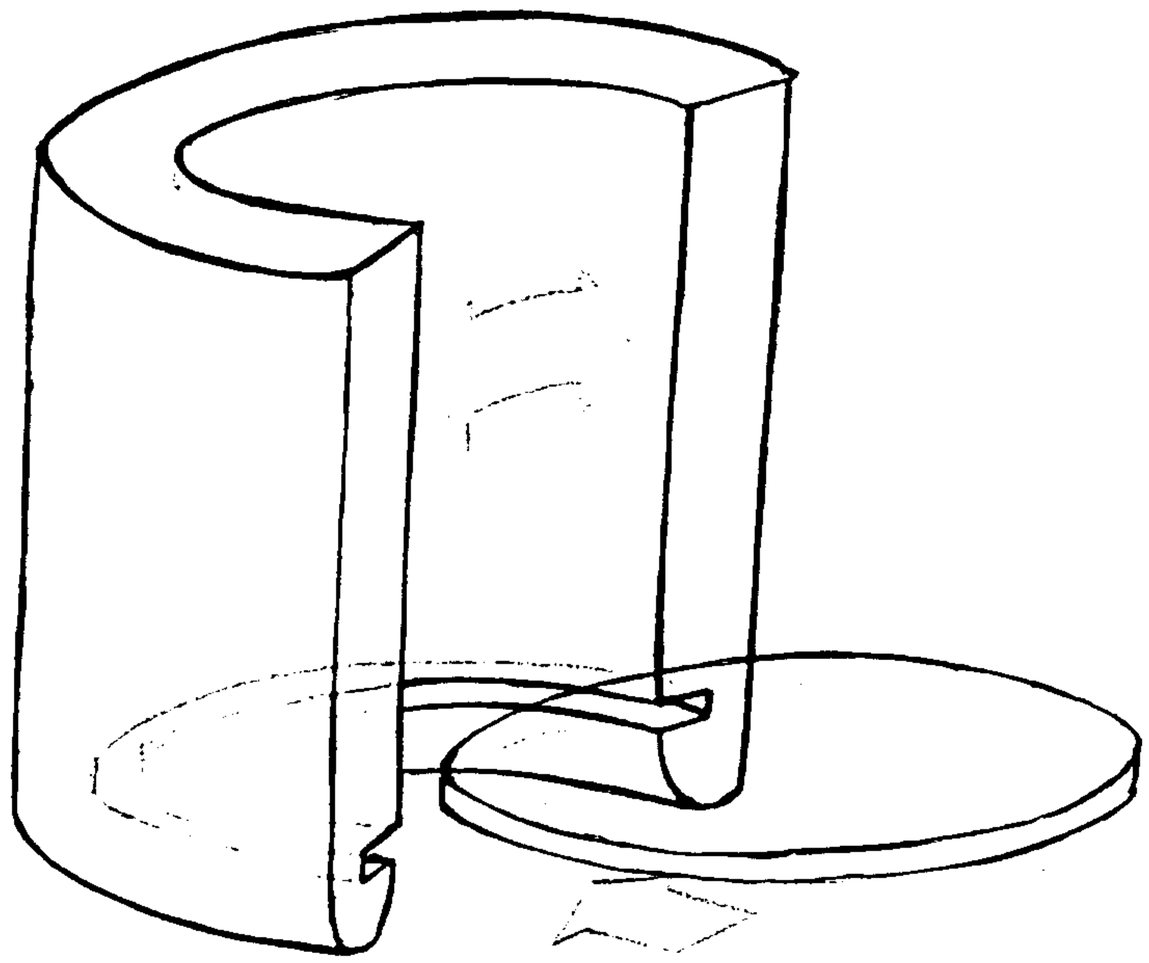


Fig. 2

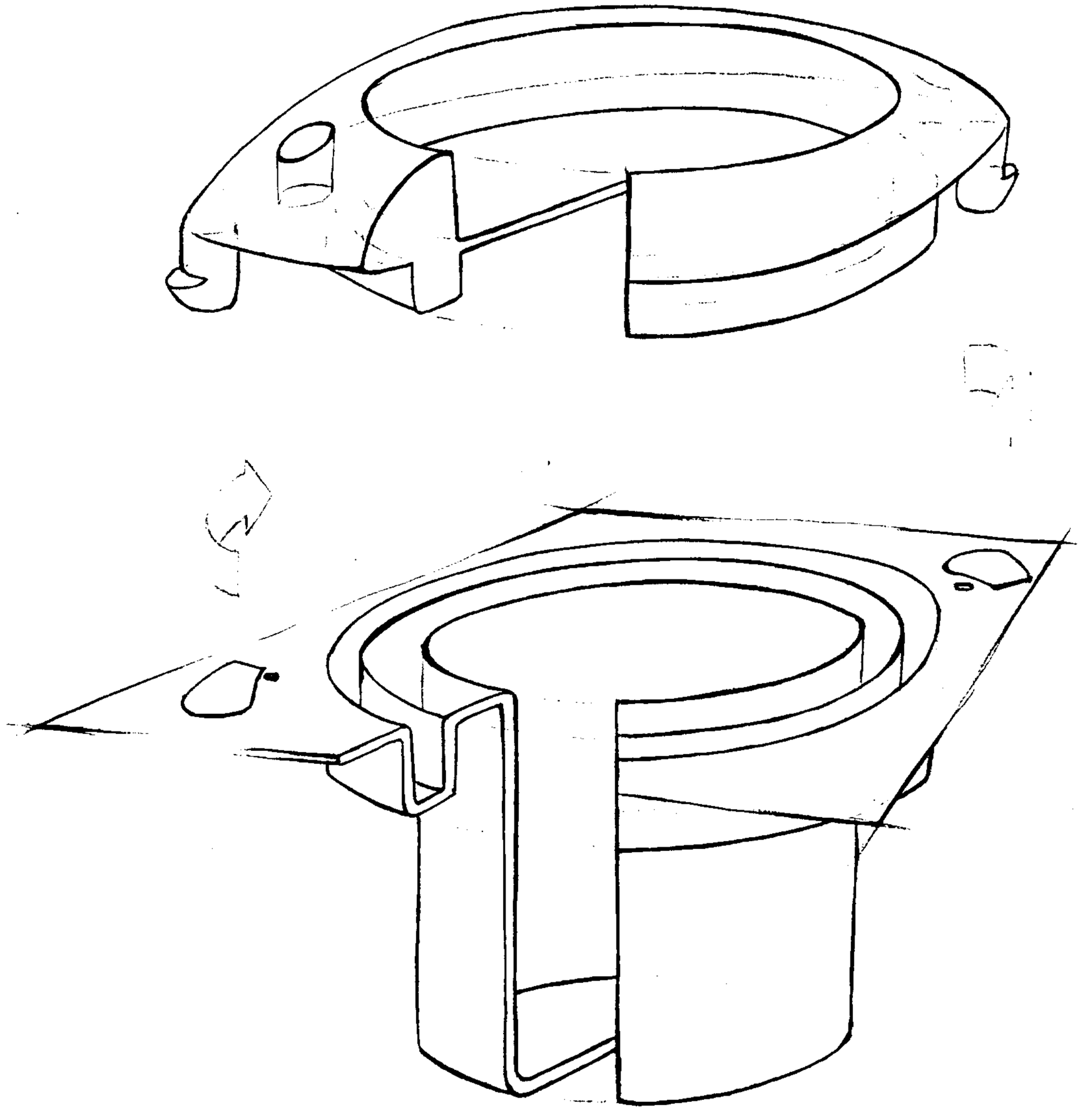


Fig. 3

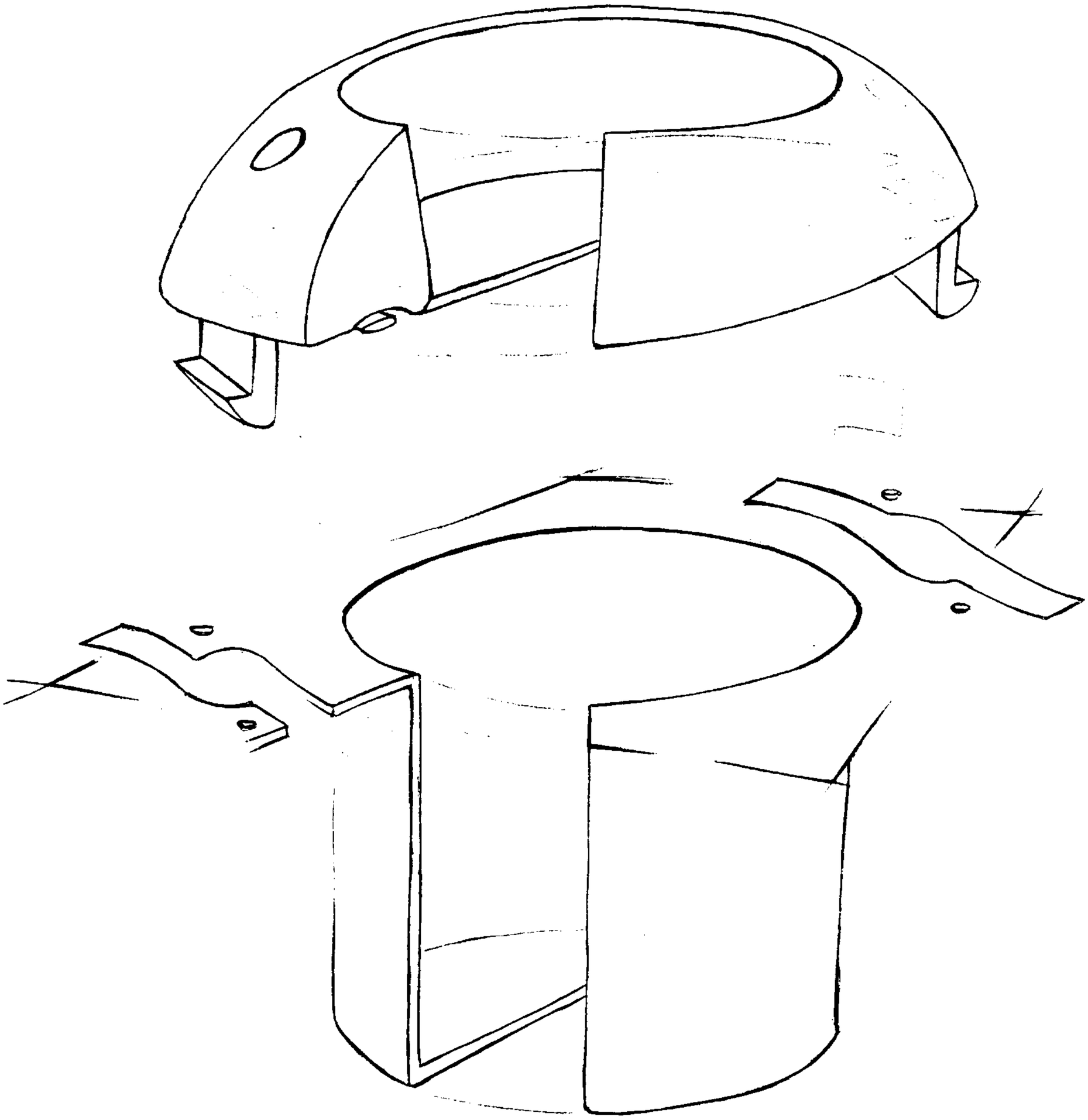


Fig. 4

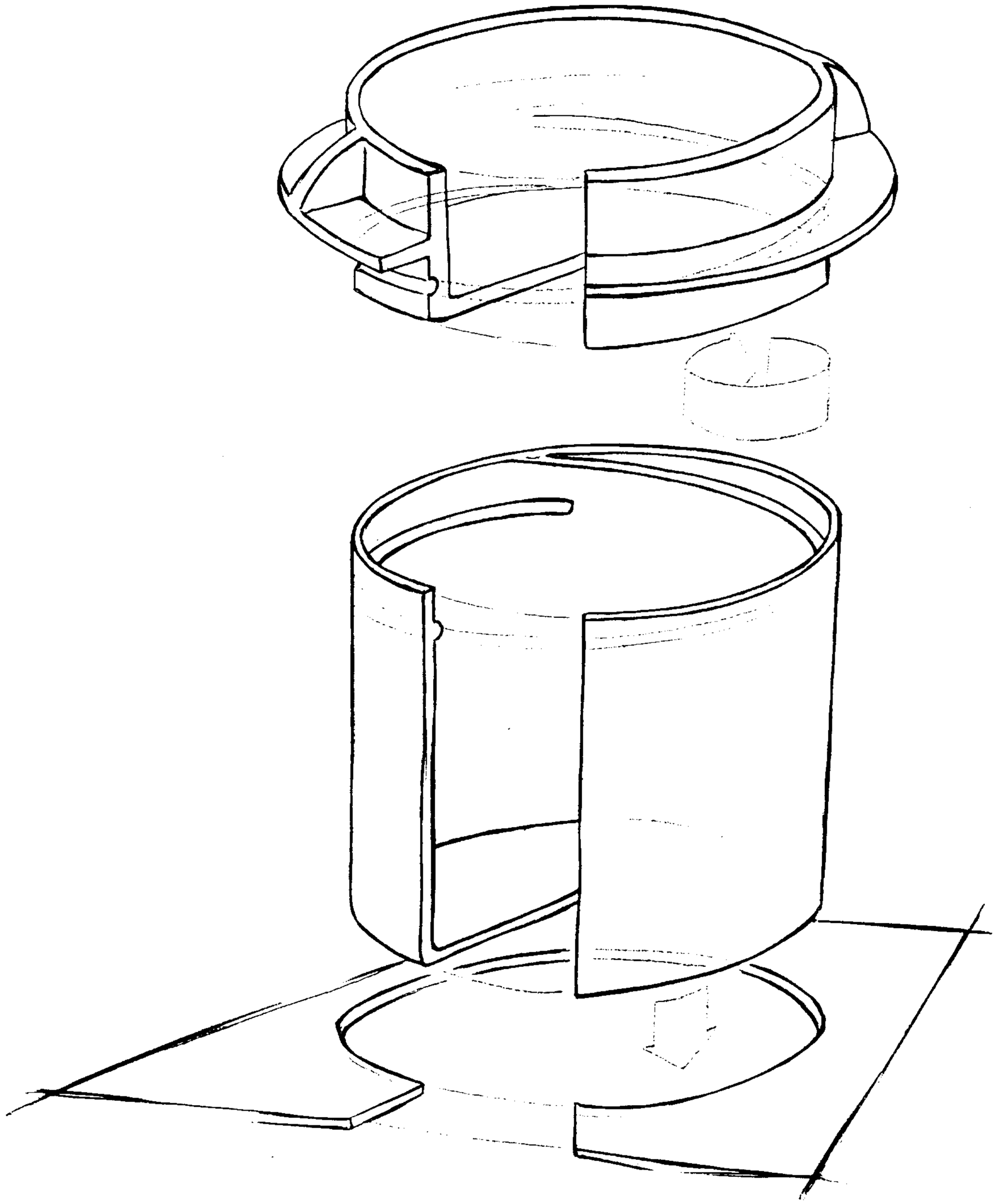


Fig. 5

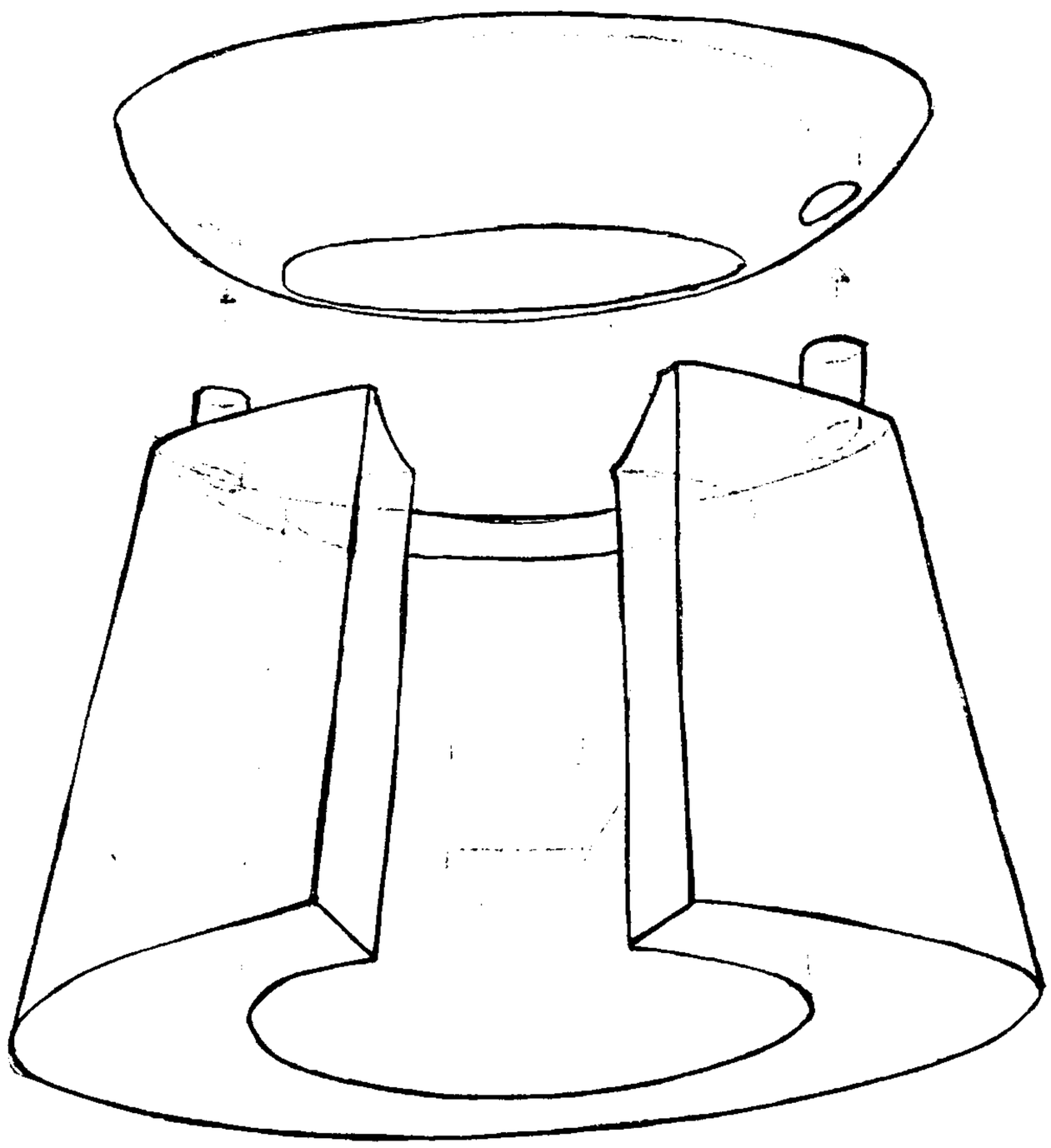


Fig. 6