United States Patent [19]

Sado

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[54] **SILICONE STOPPER FOR A STERILE CONTAINER**

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[52] **U.S. Cl.** **215/47**

[51] **Int. Cl.** **B65d 39/00**

[58] **Field of Search**.....215/47, 56, 38; 220/44 A

[56] **References Cited**

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Attorney—Harry C. Bierman, Jordan B. Bierman and Bierman & Bierman

[57] **ABSTRACT**

An otherwise closed sterile container is closed by inserting into the mouth thereof a removable plug. The plug is of silicone rubber having an open cell cellular structure. Sterility of the container is maintained. Moisture evaporation therefrom is reduced.

7 Claims, 4 Drawing Figures

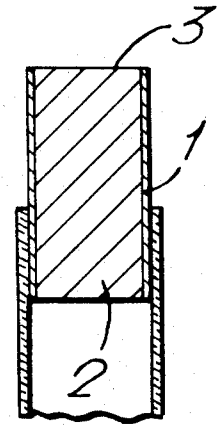


Fig. 1.

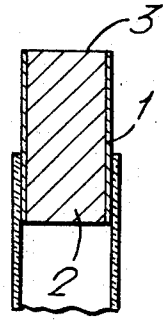


Fig. 2.

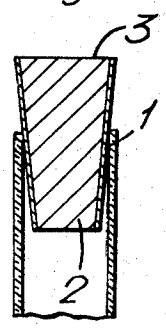


Fig. 3.

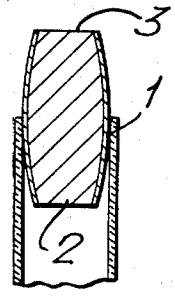
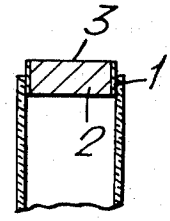


Fig. 4.



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SILICONE STOPPER FOR A STERILE CONTAINER

This invention relates to plugs to be fixed into the mouths of vessels used for culturing microbes or for rearing animals in a germ free environment.

Hitherto, plugs of cotton wool were used to plug the mouths of vessels used for culturing microbes or viruses or for rearing animals in a germ free environment. Plugs of cotton wool have several advantages. They have good air-permeability and superior resistance to the high temperatures at which sterilization is generally conducted. However plugs of cotton wool cannot stand up to repeated used. Further, much labor is required in shaping the cotton wool into plug form. Still further, it has recently become difficult to obtain good quality cotton wool and its price has gone up considerably. Stoppers of plastic or rubber, having an air-permeable structure, and plugs of urethane having an open cell structure in which all the cells are practically interconnecting have been developed as replacements for the cotton wool plugs. However the stoppers of plastic or rubber are disadvantageous in that they possess poor elasticity. This makes it difficult to obtain a close fit between the stoppers and the mouths of the vessels. Urethane plugs have poor heat resistance. Thus the sterilization of urethane plugs has to be conducted in a low-temperature gas or in steam under low pressure.

The plugs of the present invention are comprised of a silicone rubber having an open cell cellular structure and are free of the above referred to disadvantages. This silicone material is extremely stable to heat. Thus the plugs of the present invention can be sterilized at a high temperature. Such high temperature sterilization is not possible with prior art urethane plugs. Furthermore the plugs of the present invention can withstand repeated use, possess the necessary degree of permeability required in culturing microbes and viruses, and afford a much lower moisture evaporation rate than the prior art plugs. The plugs of the present invention help to prevent the culture medium from drying up or the liquid culture medium from undergoing changes in concentration.

To give a more detailed description of the plugs of the invention, reference is made to the attached drawings, of which

FIG. **1** is a plan view in longitudinal section of a column-shaped plug,

FIG. **2** is a plan view in longitudinal section of a truncated cone-shaped plug,

FIG. **3** is a plan view in longitudinal section of a barrel-shaped plug, and

FIG. **4** is a plan view in longitudinal section of a disc-shaped plug.

In FIGS. **1–4**, **1** denotes the outer skin of the plug. Outerskin **1** has a smooth surface. This facilitates an easy fit of the plug into the mouth of a vessel, (not shown). **2** denotes a layer of open cell cellular material and **3** denotes the cut surface of the cellular material **2**.

The plug of the present invention can be shaped as shown in the figures. Alternatively it may be cone-shaped, spherical, pillar-shaped, and the like. The shape should be selected in accordance with the use to which the plug is to be put.

The open cell cellular material **2** is prepared for example by the following procedure:

a. A mixture of 100 parts by weight of a diorganopolysiloxane gum with from 10 to 100 parts by weight of a silica filler such as diatomaceous earth and aerosil, from 0.05 to 15 parts by weight of a blowing agent, such as azobisisobutyronitrile, dinitroso pentamethylenetetramine, N,N'-dimethyl N,N'-dinitroso terephthalamide, and p,p' oxy bis (benzene sulfonyl hydrazide), and from 0.1 to 10 parts by weight of a curing agent, such as organic peroxide, e.g., benzonyl peroxide, diteritiary butyl peroxide, 2,4-dichlorobenzoyl peroxide, dicumyl peroxide, and tertiary butyl perbenzoate, is kneaded on a roller mill.

b. The kneaded mixture is then put through an extruder to prepare for molding.

c. The extruded kneaded mixture is then placed in a metal mold where it is heated at a temperature of from 200° to 400°C for blowing and curing

d. After completion of the blowing and curing step the mixture is then subjected to a post-curing at about 200°C in an air-circulating oven.

It should be noted that the open cell cellular material of the present invention may also be obtained by mechanical process from a known silicone sponge which has a closed cell structure in which its cells are non-interconnecting with one another.

In preparing the plug of the present invention, the surface skin **1** and the layer of open cell cellular material **2** may both be molded simultaneously. However, if it is preferred, the surface skin **1** and the layer of cellular material **2** may be prepared separately and then they may be joined together. Alternatively, after the surface skin **1** is shaped to the desired shape, the kneaded mixture may be made to foam in it. Usually the surface skin **1** is prepared with a smooth surface. However, if necessary, it can be finished unevenly. This makes it more difficult to remove the plug from the mouth of the vessel.

There is no special requirement with respect to the thickness of the surface skin **1** of the plug. It may be selected in accordance with the shape and size of the vessel mouth into which the plug is to be inserted. The degree of expansion, the hardness, and the air-permeability of the layer of cellular material **2** are also suitably selected depending upon the kind of microbe employed and the culture conditions.

The details of the invention will be further described in several examples in which the plugs of the present invention were used.

Example 1

750 parts by weight of dimethylpolysiloxane gum were kneaded on a roll mill together with 270 parts by weight of diatomaceous earth. 1.5 parts by weight of azobisisobutyronitrile and 1.9 parts by weight of benzoyl peroxide were added to 100 parts of the kneaded mixture, and the mixture was once again kneaded uniformly on a roll mill. After this second kneading the mixture was placed in a column-shaped metal mold having a diameter of 20 mm and a height of 30 mm and was heated at 250°C for 10 minutes under a pressure of 300 g/cm², so that the mixture might be blown. This was followed by post-curing at 200°C for 5 hours in an air-circulating oven. A plug of silicone rubber as shown in FIG. **1** was obtained. The plug had an open cell structure and was blown 380 percent of the original size.

The column-shaped plug of silicone rubber thus prepared was then dipped in hot water having a temperature of from 75° to 80°C, dehydrated, and sterilized for 3 hours at a temperature of 170° to 180°C. 10 g of sterilized water was placed in a sterilized test tube which measured 19 mm in inside diameter and 180 mm in height. The above prepared plug was fixed into the mouth of the test tube. The test tub was then permitted to stand for 6 months in an unsterilized atmosphere and at temperature of from 30° to 40°C. At the end of the 6 month period the amount of water evaporated out of the test tube proved to be 1.2 g. No invasion of germs was observed. The size, the shape and the elasticity of the plug was found to have undergone hardly any change.

As a comparison, the procedure of Example 1 was repeated, only a plug of cotton wool was utilized instead of the plug of the present invention. The result proved that although no invasion of germs was observed, the amount of water evaporated was 2.8 g.

On the other hand, when a column-shaped plug of commercially available urethane, having an open cell structure and measuring 20 mm in outer diameter and 30 mm in height, was fixed into the mouth of a sterilized test tube of the size given above, said test tube containing 10 g of sterilized water, and was permitted to stand for 2 weeks at temperature of from 50° to 60°C, the amount of water evaporated proved to be 2.3 g. Further it was noted that the column-shaped plug had lost its elasticity and could not recover its original shape and size.

Example 2

A truncated cone-shaped plug of silicone rubber (Cf. FIG. 2), 21 mm in upper diameter and 17 mm in lower diameter, and 30 mm in height, and having an open cell structure blown 400 percent the original size, was sterilized and fixed into the mouth of a sterilized test tube as described in Ex. 1 and containing 10 g of sterilized water. The plugged test tube was then permitted to stand for 2 weeks at a temperature of from 50° to 60°C. At the end of the 2 week period the amount of water evaporated proved to be 0.9 g and the size, the shape and the elasticity of the plug were found to have undergone hardly any change.

Example 3

Microbes were cultured in test tubes. One group of test tubes were plugged with plugs of open cell cellular silicone rubber, like the one employed in Example 1. The other group of test tubes were plugged with the prior art plugs of cotton wool. The results obtained were as given below. The growth rates of the microbes are given by the values of intensities of the wave length, 660 mμ, as measured on a photoelectric colorimeter, and the values are the averages of those obtained with 10 test tubes.

	Growth rate				Reduction in amount of liquid culture medium
Culture method	Stationary culture		Shake culture		Shaken for 7 days at 28°C and then permitted to stand
Kind of microbes	Pseudomonas ovalis	Escherichia coli	pseudo monas ovalis	escherichia coli	for 6 days at 37°C.
Plugs of cotton wool	0.238	0.163	1.15	0.98	0.86 cc
Plugs of open cell cellular silicone rubber	0.245	0.162	1.10	0.98	0.39 cc

Note)

Culture medium: Compositions		Gravy 0.3% Peptone 0.5% Glucose 1.0% Water...rest
pH		7.0
Amount		10 cc
Culture condition:		28°C / 22 hours
Shaking condition:		300 shakes / min. 25 mm – stroke
Test tubes:		Inside diameter; 18–19 mm Inserted length of the plugs: ca. 25mm
Size of plugs:		Plugs of cotton wool ca. 40mm in length
	Plugs of open-cell cellular silicone rubber	45 mm in length & ca. 20 mm in diameter

What is claimed is:

1. A plug for maintaining sterility and reducing moisture evaporation from an otherwise closed sterile container having a mouth, said plug being adapted for removable insertion into said mouth to close same, said plug being comprised of silicone rubber having an open cell cellular structure.

2. The plug as claimed in claim 1 wherein said silicone rubber is obtained by mechanical process from a closed cell cellular material.

3. The plug as claimed in claim 1 wherein said plug has an exterior surface which is smooth.

4. The plug as claimed in claim 3 wherein said plug has an exterior surface which is uneven whereby removal of the inserted plug from said mouth is rendered more difficult.

5. The plug as claimed in claim 1 wherein said container contains a microbe culture.

6. The plug as claimed in claim 1 wherein said container is an animal rearing chamber.

7. The plug as claimed in claim 1 wherein said plug is permeable to air.

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