

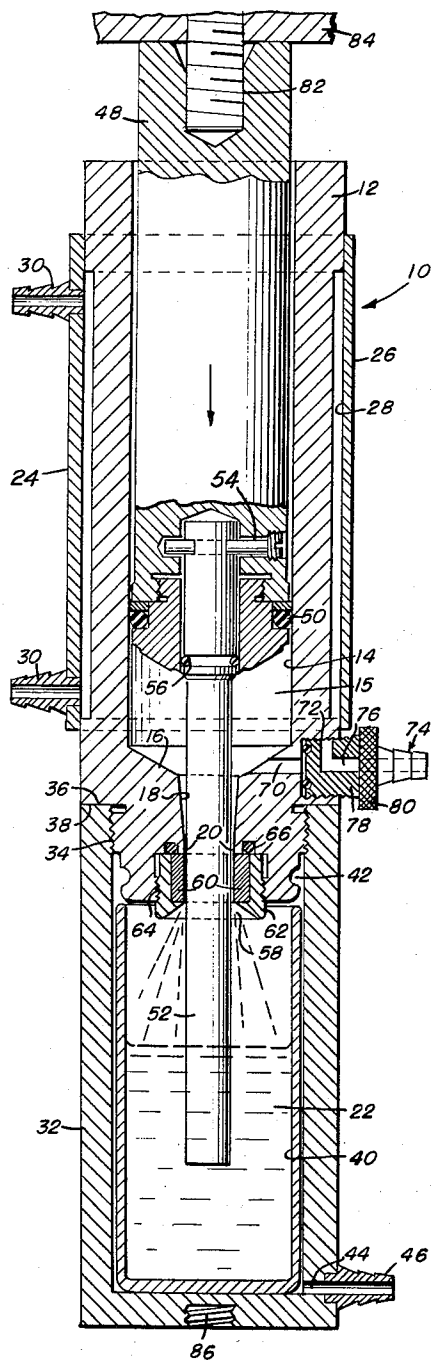
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HYDRAULIC TISSUE HOMOGENIZER

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HYDRAULIC TISSUE HOMOGENIZER

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This invention relates to an explosive pulverization device. More particularly it relates to a device based on pressure release principles and adapted to homogenize animal and plant tissues with greater ease and reproducibility than heretofore possible.

In particular, the instant invention relates to an apparatus for controlled release of the cellular components from various tissues rapidly and at low temperatures.

The investigator who wishes to disperse tissues in a liquid medium often has one of three objectives in mind. He may desire to completely comminute the tissues and cells preliminary to some isolation procedure. Alternatively he may wish to control the degree of tissue breakdown so that some particular cell inclusions remain intact. Finally he may desire to produce homogenates very rapidly and in a reproducible fashion for purposes of metabolic investigations.

Ideally the same device should be capable of producing only whole cells, or alternatively, the intact products of whole cells (i.e., nuclei, mitochondria, and microsomes), or alternatively the dispersed contents of nuclei plus the other inclusions. Moreover the desired results should be accomplished rapidly, at low temperatures, and should yield metabolically active products.

The prime objects of this invention is to provide a device for uniformly comminuting tissue materials.

A further object of this invention is to provide a device which can prepare uniform dispersions of tissue fragments in liquid media.

Further objects and the advantages of the instant invention will be apparent from the description which follows.

Advantageously, the homogenizer of the instant invention provides for application of a high pressure upon a confined liquid suspension of tissue matter. A piston which applies the pressure forces the suspension through a small annular orifice into a region of low pressure. The sudden pressure drop realized as the suspension passes through the orifice causes comminution of the tissue to a degree predetermined by the orifice size.

Referring now to the drawing wherein there is illustrated in cross section and semi-diagrammatically a preferred embodiment of the apparatus comprising the instant invention, the device as a whole is shown at 10.

Device 10, hereinafter called the homogenizer, comprises an elongated cylindrical vessel or container 12, its chamber 14 being adapted to hold a roughly ground-up tissue suspended in a buffer liquid, shown at 15. As illustrated in the drawing, chamber 14 has an open top and a tapered bottom outlet 16. Tapered outlet 16 terminates with a vertical downspout 18 of slightly decreasing diameter, top to bottom. The drawing exaggerates the taper on downspout 18 for ease of viewing. Tapered downspout 18 terminates in a nozzle 20. Desirably vessel 12 may be provided with a cooling jacket 24 to chill the contents. For ease of construction and because of the high pressures sustained by homogenizer 10 during operations, vessel 12 is preferably made of a strong corrosion

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resistant material such as stainless steel. Forming vessel 12 out of metal allows certain advantages; accurate machining of chamber 14 is facilitated. Also cooling jacket 24 may then be a metallic sleeve 26 shrunk-fit around a recessed portion 28 in the outer wall of vessel 12. The inlet and outlet taps 30 provided in cooling jacket 24 can be built into the sleeve.

The base of homogenizer 10 is a cup-shaped member 32 suitably constructed to be secured underneath chamber 14, e.g., by a threaded connection 34. An important aspect of the interconnection between base 32 and vessel 12 lies in the need for accurate alignment. Desirably, flat annular opposing surfaces 36, 38 are provided on vessel 12 and base 32, respectively, to give a large area for actual contact so that stresses incurred during operation are transmitted through flat surfaces 36, 38 rather than through the threaded connection 34. If desired, interrupted threads may be used for connection 34 so that the operator can more easily thread vessel 12 and base 32 into contact at opposing surfaces 36, 38.

Ordinarily a plastic, glass or metallic retainer cup 40 will be loosely positioned inside of base member 32. However, for the occasions when contact of the homogenizate with air should be minimized, an annular groove 42 is provided on the bottom of vessel 12. A collapsible receiver such as a rubber balloon may be hung from groove 42 to receive the homogenizate issuing through nozzle 20. Base 32 is provided with a vent passage 44. A tap 46 can be built into vent passage 44 to allow connection with a rubber or plastic hose.

Loosely disposed in the upper portion of chamber 14 is a piston 48. One or more gaskets like O-ring 50 should be provided in recessed portions of piston 48 to insure a uniform contact between the piston 48 and the wall of chamber 14. The gasket 50 barely touches the wall of chamber 14 when piston 48 is at rest. Application of a moving force to piston 48 causes the O-ring gasket 50 to expand, completely sealing the annular space between piston and chamber wall against leakage from chamber 14 and consequent loss of chamber pressure.

Removably attached to piston 48 and depending from the interior thereof is a center rod 52. This center rod 52 is secured to the body of piston 48, suitably through at an offset pin 54. One or more such pins may be employed. Alternatively, other suitable modes of interconnection may be employed, e.g., an Allen head screw. In any event center rod 52 is detachable by release of securing element 54 so that the rod may readily be slipped out the bottom of piston 48. In removing and replacing center rod 52, due care should be taken to avoid damaging the gasket 56 (one or more O-rings) employed to maintain center rod 52 accurately positioned relative to the body of piston 48. Gasket 56 serves also to prevent leakage from chamber 14 to the hollow interior region of piston 48 where center rod 52 is disposed; gasket 56 thereby prevents loss of pressure from chamber 14 and permits atmospheric pressure to exist at the top extremity of center rod 52 during operation of the homogenizer. Atmospheric pressure always exists at the discharge end of nozzle 20, and consequently at the bottom extremity of center rod 52. As a result comparatively little stress is placed on offset pin 54 by operation of the homogenizer.

It is critically important that center rod 52 be of substantially uniform dimensions (plus or minus 0.0001 inch) throughout the portion of its length which passes downwardly through nozzle 20. As shown in the drawing, a relatively loose fit of the center rod 52 in piston 48 is actually desirable since downward movement of piston 48 then allows or permits a very slight lateral movement of the rod. This slight lateral movement amounts to a pumping action which facilitates smooth

and efficient ejection of homogenate through the annular orifice 58 spaced between center rod 52 and nozzle 20.

The presence of an accurately sized annular orifice 58 is critical in the device of the instant invention. To insure uniformity and a constant orifice width, the center rod and nozzle must be accurately dimensioned. For best results it is imperative that the center rod be machined to a roundness and constant diameter within ± 0.0001 inch.

The materials of construction for center rod 52 and nozzle 20 must be capable of maintaining their dimensional accuracy despite the stresses and strains imposed on these elements by operation of the homogenizer. Suitably, center rod 52 can be fabricated from stainless steel. The nozzle material, however, should be dissimilar from the center rod in order to prevent seizing at minimum orifice widths. Thus nozzle 20 can comprise a finely ground carbide (e.g., tungsten, boron, chromium, etc.) insert 60 mounted in a threaded housing 62 which in turn is threaded into a central recess 64 in the bottom-most portion of vessel 12. A compressible seat is provided by O-ring 66 in order to prevent leakage around the nozzle. A carbide insert 60 is capable of being ground with diamond dust and polished to an inside diameter accurate within ± 0.0001 inch, giving nozzle 20 the desired uniform and predetermined inside diameter.

The specific orifice size depends on the diameters of rod 52 and of nozzle 20. The actual width of the annular orifice at room temperature should be pre-selected at a level between 6 and 130 microns (0.00025 to 0.005 inch) by appropriately forming nozzle 20 and center rod 52. To attain a desirable flexibility of operation, orifice size may be changed by substitution of different nozzle inserts 60 and, or, alternatively different center rods 52. Preferably center rod 52 is changed. To this effect, a set of appropriately sized center rods are ordinarily provided with each homogenizer 10 so that specific orifice widths of 6 microns, 130 microns or each of several intermediate widths can be employed as desired.

The drawing further illustrates certain optional features which have been found useful. At the lower end of chamber 14 a valved outlet passage 70 may be provided. Outlet passage 70 terminates at a larger threaded opening 72 so that a vacuum release valve 74 can be built directly into the body of vessel 12. A suitable port (not shown) provided in the threaded opening 72 is brought into or out of alignment with passage 76 in the valve 74 by rotating or turning the valve body 78. A knurled knob 80 is provided to facilitate rotation of valve body 78 into the open or closed position.

At the top of piston 48 a threaded recess 82 is provided so that the piston may be secured to some suitable mechanism for forcing the piston down. The drawing illustrates the connection to upper platen 84 of a Carver laboratory press. A similar threaded recess 86 is provided on the underside of base member 32.

The operation of the homogenizer is as follows. A tissue suspension can be introduced into the cylinder in three ways:

(1) The base 32 is separated from the vessel cylinder 12, and piston 48 is pulled back so as to withdraw center rod 52 from the orifice 58. The mixture is then poured through the nozzle 20 directly into the inverted chamber 14; the piston is then pushed up (with the cylinder still inverted) until the center rod obstructs the nozzle opening.

(2) The piston 48 with its center rod 52 is separated from the cylinder, the opening at the bottom of the nozzle 20 is closed with a stopper, and the tissue mixture is poured into the open top end of the cylinder. The piston is then replaced and pushed past the O-ring 50, the cylinder is inverted, the stopper is cautiously removed (or vacuum-release valve 74 is opened), and the

piston is pushed so that center rod 52 closes the nozzle opening.

(3) The tissue mixture containing fragments no larger than 2 to 3 mm. can also be introduced through the vacuum-release valve 74. In this case, homogenizer 10 is completely assembled and threaded into the Carver press with the head of piston 48 resting at the bottom of chamber 14. The vacuum-release valve 74 is opened by one turn, counter clockwise of its knurled knob 80. A Tygon or rubber tube is then attached to the vacuum release valve, the open end of the tube being immersed in the tissue suspension. The suction created by pulling the homogenizer downward draws the tissue mixture into the bottom of chamber 14. Vacuum-released valve 74 is then closed.

Regardless of how chamber 14 is filled, the assembled apparatus containing the tissue mixture is placed in a device adapted to force piston 48 down, e.g., in a Carver press with its platens apart. The lower platen is raised slightly until the homogenizer piston almost touches the upper platen. The proper size bolt is manually screwed into recess 82 of piston 48 to insure that homogenizer 10 is centered in the press. The piston is now rapidly pushed into the cylinder. This application of force to piston 48 places the suspension in chamber 14 under a high pressure, forcing the suspension out of chamber 14 through annular orifice 58. A sudden release of pressure occurs at the instant of passage through orifice 58 to cup 40 effecting an explosive pulverization or comminution of the tissues. One excursion of piston 48 (2 to 3 minutes) is sufficient to comminute the tissue to an extent determined by the size of the annular orifice, and disperse the fragments in the liquid buffer. The piston can now be readily withdrawn from chamber 14 by opening vacuum-release valve 74.

For most purposes, the homogenate is collected in the retainer cup 40 normally disposed inside base 32. Some foaming occurs upon homogenization which may result in a slight loss when the volume of the recovered homogenate is high (e.g., exceeds 50 ml. with a chamber 14 of 1.5 inch diameter). Foam loss may be avoided and air contact avoided by collecting the homogenate in a collapsed rubber bag or balloon hung from groove 42. In this way the comminution products can be completely recovered. Ordinarily comminuted connective tissue quickly floats to the top of the homogenate.

To homogenize large volumes of a tissue or cell suspension, retainer cup 40 is removed and a delivery tube is attached to vent tap 46. The homogenizer is filled by method 3, described above, and homogenization of the tissue is carried out. The homogenate drains out through vent passage 44 to the delivery tube. In this fashion, filling and homogenization can be done repeatedly without dismantling the apparatus.

In a specific homogenizer 10 built with a chamber $1\frac{1}{2}$ " in diameter the best results are achieved with at least 20 ml. of suspension. In fact, for this size homogenizer little or not comminution will result with less than 10 ml. At high dilution of tissue in buffer, e.g., 1 gm./20 ml., the greatest efficiency results. Satisfactory comminution, or homogenization, will result with as much as 2.5 ml. of buffer per gm. of tissue. Still higher tissue concentrations interfere with proper operation. In general it should be borne in mind that operation of the device depends upon the velocity of the tissue suspension passing through annular orifice 58. Accordingly the tissue suspension should be reasonably fluid.

The homogenizer of the instant invention is particularly adapted for biochemical and biological laboratory work or such temperature sensitive materials, e.g., liver tissue. By prechilling the homogenizer and the buffer medium, and by circulating brine in cooling jacket 24, homogenization of large quantities of tissue can be effected at near freezing point temperatures. For smaller quantities of tissue or where thermal stability is not too

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critical, one or more of these aforementioned cooling expedients can be omitted.

Whereas the invention has been described in connection with a particular embodiment, it will be understood that this is only for the purpose of explanation and that the invention is to be limited only by the spirit and scope of the appended claims.

What is claimed is:

1. A pressure release comminuting device comprising: a container adapted to hold a suspension of solid particulate matter suspended in a liquid medium, said container having a bottom outlet terminating in a nozzle; a close-fitting piston disposed in said container above the bottom outlet thereof; an elongated rod extending from said piston through said bottom outlet and nozzle opening; the dimensions of the portion of said rod extending through said nozzle being uniform, and the dimensions of said nozzle opening being uniform, whereby a small predetermined annular orifice is provided between the rod and the nozzle through which the suspension is forced when said piston is advanced toward the bottom of said container.

2. A homogenizer for pressure release of cellular components which comprises: a container adapted to hold a suspension of solid particulate matter suspended in a liquid medium, said container having a bottom outlet terminating in a nozzle; a close-fitting piston disposed in said container above the bottom outlet thereof; an elongated rod removably attached to said piston and extending from said piston through said bottom outlet and nozzle opening; the dimensions of the portion of said rod extending through said nozzle being uniform, and the dimensions of said nozzle being uniform, whereby a small predetermined annular orifice is provided between

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the rod and the nozzle through which the suspension is forced when said piston is advanced toward the bottom of said container.

3. The apparatus of claim 2 wherein a cup-shaped base member is provided, said base member and said container being attachable into an integral unit and the homogenate discharged through said orifice being collected in the cup portion of said base member.

4. The apparatus of claim 2 wherein a cooling jacket surrounds the container.

5. The apparatus of claim 1 wherein the nozzle is a separate insert made of tungsten carbide ground to an internal diameter accuracy of ± 0.0001 inch, and wherein the external diameter of the center rod is dimensioned to an accuracy of ± 0.0001 inch.

6. The apparatus of claim 1 wherein a set of interchangeable rods are provided each rod being of different uniform dimensions whereby an annular orifice ranging from between about 6 to about 130 microns at room temperature may be preselected.

7. The apparatus of claim 1 wherein a valved side opening is provided in said container adjacent said bottom outlet, said valved side opening being adapted to release the vacuum generated in said container by movement of said piston away from the bottom of said container.

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