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Parr Cell Disruption Vessels

Cell Disruption By Nitrogen Decompression

Designing and Building High Precision Laboratory Equipment for Over 100 Years



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Cell Disruption by Nitrogen Decompression

A rapid and effective way to:

- **Homogenize cells and tissues**
- **Release intact organelles**
- **Prepare cell membranes**
- **Release labile biochemicals**
- **Produce uniform and repeatable homogenates without sub-jecting the sample to extreme chemical or physical stress.**

A Widely Accepted Method

Cell disruption by rapid decompression from a pressure vessel has been used for many years by investigators who wanted to overcome the limitations imposed by other cell disruption procedures. Although the technique is not new, interest in the decompression method and many new applications for it have grown rapidly following the introduction of convenient pressure equipment such as the Parr Cell Disruption Vessel.

Many Applications

The nitrogen decompression method is particularly well suited for treating mammalian and other membrane-bound cells. It has also been used successfully for treating plant cells, for releasing virus from fertilized eggs and for treating fragile bacteria. It is not recommended for untreated bacterial cells, but this restriction can be eliminated by using various pretreatment procedures (p. 7) to weaken the cell wall. Yeast, fungus, spores and other materials with tough walls do not respond well to this method.

How It Works

The principle of the method is quite simple. Large quantities of nitrogen are first dissolved in the cell under high pressure within a suitable pressure vessel. Then, when the gas pressure is suddenly released, the nitrogen comes out of the solution as expanding bubbles which stretch the membranes of each cell until they rupture and release the contents of the cell.

Why It Is So Effective

It's a gentle method. Although sometimes referred to as "explosive decompression," nitrogen decompression is actually a gentle method for homogenizing or fractionating cells since the chemical and physical stresses which it imposes upon the sub-cellular components are held to an absolute minimum. It is much more protective of delicate enzymes and organelles than ultrasonic and mechanical homogenizing methods. In fact, it compares favorably to the controlled disruptive action obtained in a Teflon and glass mortar and pestle homogenizer, but it does the job faster and more uniformly, with the added ability to treat large samples quickly and conveniently.

There is no heat damage. While other disruptive methods depend upon friction or a mechanical shearing action which generates heat, the nitrogen decompression procedure is accompanied by an adiabatic expansion which cools the sample instead of heating it. In addition, the entire cycle can be conducted at low temperature by pre-chilling or by operating the vessel in an ice bath. The vessel can also be filled with ice to keep the sample cool during the processing period.

There is no oxidation. The blanket of inert nitrogen gas which saturates the cell suspension and the homogenate offers excellent protection against oxidation of any labile cell components. Although other gases: carbon dioxide, nitrous oxide, carbon monoxide and compressed air have been used in this technique, nitrogen is preferred because of its non-reactive nature and because it does not alter the pH of the suspending medium. In addition, nitrogen is preferred because it is generally available at low cost and at pressures suitable for this procedure.

Any suspending medium can be used. The suspending medium can be chosen for its compatibility with the end use of the homogenate and without regard for its adaptability to the disruptive process. This offers great flexibility in the preparation of cell suspensions and produces a clean homogenate which does not require intermediate treatment to remove contaminates which might be introduced when using other disruption methods.

Each cell is exposed only once. Once released, subcellular substances are not exposed to continued attrition which might denature the sample or produce unwanted damage. There is no need to watch for a peak between enzyme activity and percent disruption.

The product is uniform. Since nitrogen bubbles are generated within each cell, the same disruptive force is applied uniformly throughout the sample, thus ensuring unusual uniformity in the product. Cell-free homogenates can be produced.

It's Easy To Apply

Use any sample size. Cell disruption by this method is independent of sample size or concentration. Any size sample from a few cc's to five hundred can be treated equally well in a Parr Cell Disruption Vessel with excellent recovery of the starting material. In addition, a wide variety of materials can be treated with the opportunity for scale-up work where labile cell components or organelles are involved.

Easy to control. The degree of cell fractionization is easily controlled by adjusting the nitrogen pressure. High pressures which dissolve large quantities of nitrogen within the cell usually produce total homogenization. Or, moderate pressures can be employed to reduce the disruptive forces and thus release nuclei, active mitochondria and other organelles intact. Operating conditions can also be adjusted to homogenize suspensions of subcellular components such as nuclei and mitochondria that are normally difficult to disrupt because of their small size.

Special skills are not required. The few simple steps required to operate the Parr vessel are easily learned. After operating conditions have been established, uniform and repeatable results can be obtained from run to run, usually within less than twenty minutes – even with large samples.

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Cell Disruption Vessels in Five Convenient Sizes

Parr stainless steel vessels for processing cell suspensions by the nitrogen decompression method are made in several sizes with full opening heads and self-sealing closures which can be handled easily on any laboratory bench without special tools or fixtures. Each vessel has two valves and a pressure gage: one valve for charging with nitrogen and the other for withdrawing the homogenate and discharging it through an attached delivery tube. All of the fittings as well as the vessel itself are made of stainless steel with polished surfaces for good corrosion resistance and freedom from contamination. The individual parts are easily cleaned and can be thoroughly sterilized.

Designs For Large And Small Samples

These vessels are made in five different sizes to accommodate samples ranging from 0.5 ml to 5 liters, as listed in the table on page 4. The maximum charging capacity for each vessel is limited to two-thirds of the internal volume of the vessel, but smaller samples can be treated in any of these vessels by simply placing the sample in a beaker or test tube and positioning it under the dip tube within the vessel.

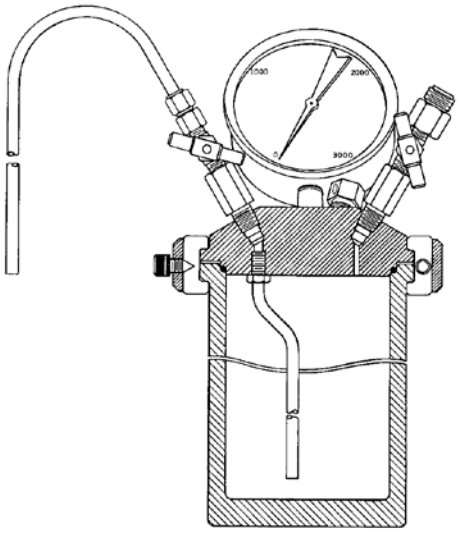
The 4635, 920 ml vessel is the general purpose model with a capacity for treating samples ranging in size from a few milliliters up to 600 ml.

The 4639, 45 ml vessel is designed specifically for small samples from 30 ml down to less than one milliliter. It has a tapered bottom which drains directly into the discharge valve, ensuring complete recovery of all of the sample.

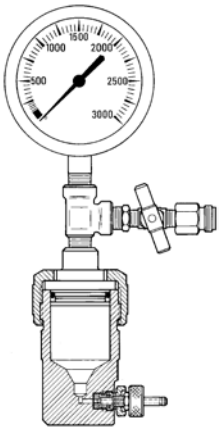
The larger vessels, 4636, 4637, and 4638, are intended for large volume applications. Users are urged to run preliminary experiments in the 4635 vessel to confirm the suitability of the procedure before scaling up to these larger sizes.



Model 4635 and Model 4639 Parr Cell Disruption Vessel



Model 4635



Model 4639

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Easy Access To Vessel Chamber

Each of these vessels, except the small 45 ml size, has a split-ring closure – an exclusive Parr design which allows the vessel to be opened or closed easily without disturbing any of the fittings or connecting lines attached to the head. In this closure two ring sections slide into place from the sides of the vessel to lock the head in position while a self-sealing O-ring maintains a tight seal at all pressures. The ring sections are secured by a steel retaining band which is raised from the bottom of the vessel and anchored with a single, hand-tightened screw.

On the small, 45 ml vessel, a firm closure is obtained by simply turning down a knurled cap until it is hand tight. No wrenches or fixtures are required.

Safety Protection

All Parr Cell Disruption Vessels are designed to accommodate the full 2200 psig available from a commercial nitrogen cylinder. If higher pressures should accidentally develop, the 920 ml and larger vessels are protected by a safety rupture disc which will burst at approximately 3000 psig, well below the pressure at which any parts of the vessel would fail. A 3000 psi pressure gage and a 3000 psig rupture disc are standard, but other gages and discs with lower pressure ratings can be furnished on special order.

Includes Nitrogen Filling Connection

An 1831 nitrogen filling connection furnished with each vessel provides all of the fittings needed to fill the vessel from a commercial nitrogen cylinder. The 1831 connection consists of a control valve with a standard CGA-580 coupling for attachment to a nitrogen cylinder, a tank pressure gage and a flexible Nylon pressure hose for connection to the vessel inlet valve. Extra O-rings for the vessel head are included with each vessel.

Custom Modifications

Most cell disruption procedures can be handled readily in the 920 ml, 4635 vessel with its customary fittings and attachments, yet applications may arise in which it will be desirable to modify a vessel to meet special requirements. If initial studies show a need for modifications, the user can return any vessel to the factory for such changes or additions as may be needed.

Changes sometimes requested include:

Multiple sample capacity. Up to three additional dip tubes and discharge valves can be installed in any vessel (except the 45 ml size). With these additional outlets and appropriate sample holders, as many as four samples can be processed independently and simultaneously in a single unit.

Special valves. Valves with either larger or smaller orifices can be provided. Users processing large quantities of materials that have a tendency to plug the orifice of the standard throttling valve may want to substitute either a larger needle valve or a ball valve to permit faster flow rates or to avoid stoppage. Fine metering valves with smaller orifices and very precise control capabilities may be helpful when treating samples that must be processed under very exact and repeatable conditions. Such valves may also be desirable when working with very small samples.

Size conversion. Since cylinders for the 920 and 1850 ml vessels are interchangeable and both vessels use the same head and closure, any user of a 4635 vessel can increase the capacity of his unit to the 4636 size by simply purchasing a deeper cylinder (599HC22) and a longer dip tube (591HC2). A similar conversion can also be made from the one gallon vessel to the two gallon size for users interested in vaccine production or in similar large volume applications. These larger vessels can also be equipped with vigorous internal agitators to speed equilibrium times when processing large volumes of material.

Inquiries concerning any of these modifications should be directed to the factory.

Ordering Information

Orders for these vessels should specify the catalog number and internal volume of the vessel as shown in the following table. Each vessel will be made of an AISI 300-series stainless steel and equipped with a 0-3000 psi pressure gage and 1831 nitrogen filling connection, unless other construction and other fittings are specified.

Parr Cell Disruption Vessels

Catalog Number	*Internal Volume*	*Maximum Sample Size*	*Maximum Working Pressure, psig*	*Inside Diameter Inches*	*Inside Depth Inches*
4639	45 ml	30 ml	2200	1.50	2.25
4635	920 ml	600 ml	2200	3.75	5.10
4636	1850 ml	1200 ml	2200	3.75	10.10
4637	1 gallon	2.5 liters	2200	6.00	8.60
4638	2 gallon	5.0 liters	2200	6.00	17.20

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Operating Procedures

Operating procedures for the Parr Cell Disruption Vessel can be adjusted to suit individual requirements. The procedures described here will be suitable for most applications, but they are not intended to be either complete or restrictive. Each operator will develop refinements which produce the best results with his particular materials, while still observing the basic vessel handling instructions and safety precautions described below.

Preparing The Cell Suspension

Individual cells such as lymphocytes, leukocytes, tissue culture cells or very fragile bacterial cells will not require pretreatment. Tissues must usually be preminced to ensure that they will pass through the dip tube and discharge valve. Hand presses, mechanical homogenizers, pressing through screens or sieves, slow speed blenders and manual dissection have all been used successfully for this purpose. Tougher tissues will require finer premincing than will softer tissues. Best results will be obtained if any connective tissue which might block or plug the discharge passages are removed or finely divided at this time.

The intended use of a homogenate generally determines the composition of the suspending medium. Isotonic solutions are commonly used. Solutions with higher concentrations will tend to stabilize the nucleus and organelles. Conversely, very dilute solutions will prestretch the cells by osmotic pressure and will render them more susceptible to disruption by the disruption method.

Several investigators have reported that very small quantities of calcium chloride, magnesium acetate, or magnesium chloride added to the suspending medium will stabilize the nuclei when differential rupture is desired. Ratios of approximately 10 ml of suspending medium to one gram of wet cells are commonly used to prepare the cell suspension. Higher or lower ratios can also be used.

Charging The Vessel

Instead of pouring the cell suspension directly into the vessel cylinder, most users will find it convenient to hold the sample in a beaker or test tube which can be placed inside the larger vessels. A supplementary container is particularly useful when working with small samples. As a general rule, the capacity of the inner container should be approximately twice the volume of the suspension to be treated. Larger samples may, of course, be poured directly into the cylinder. If an inner container is used, it must be positioned so that the dip tube leading to the release valve reaches to the very bottom of the container. Plastic test tubes, centrifuge tubes and beakers work well as auxiliary sample holders. They are not only unbreakable but they also can be floated on ice water within the vessel to keep the suspension cool during equilibration and to bring the bottom of the sample holder up to the tip of the dip tube to ensure complete sample recovery.



1831 Nitrogen Filling Connection

Sealing And Pressurizing The Vessel

After placing the cell suspension in the vessel, set the head on the cylinder with the dip tube extending into the sample holder and attach the split ring cover clamp. No wrenches, holders or special tools are needed in this closing operation. The two ring sections slide into place from the side and the drop band is raised from the bottom of the vessel to encircle them. These parts are then secured by simply turning a single thumbscrew finger tight.

To pressurize the vessel, attach the filling connection to a commercial nitrogen cylinder and connect the flexible Nylon pressure hose to the vessel inlet valve. A quick disconnect fitting on the hose makes it easy to complete this connection and to remove the vessel from the filling system after pressure has been applied. A gage on the filling connection shows the pressure in the supply cylinder while a gage on the vessel head shows the pressure applied to the sample.

It should be noted that the vessel does not have to remain at a fixed location during the loading, closing and filling operations. These steps can be performed anywhere in the laboratory and the vessel can then be carried to a different location for pressure equilibration and sample recovery if the user wishes to do so.

The amount of pressure used will vary with different samples and with different disruption or homogenization procedures. Large samples will often absorb a significant amount of nitrogen, making it necessary to re-pressurize the vessel one or more times in order to maintain the desired pressure level. Also, a significant pressure drop may occur as the homogenate is released from the vessel, making it necessary to add additional nitrogen in order to treat all of the sample at the same pressure level. Such additions can be made easily using the control valves provided on the vessel head and on the filling connection.

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Equilibrium

Sufficient time must be allowed for the nitrogen to dissolve and come to equilibrium within the cells. Periods as short as five minutes may be sufficient for small samples, while longer times up to thirty minutes may be required for large samples. Stirring with a magnetic bar placed in the bottom of the vessel will accelerate this process, particularly when working with large samples; it will also hold the cells in a uniform suspension. Since the vessel is made of a non-magnetic stainless steel, the stirring bar can be driven by simply placing the vessel on a magnetic stirring plate. If cooling is required to protect the sample, the vessel can be pre-cooled before it is charged, or ice can be packed around the inner sample holder, or the vessel can be packed in ice or held in a cold room during the equilibration period.

Disruption And Collection

The actual disruption process does not occur as cells are pressurized with nitrogen. Instead, it occurs at the discharge valve at the instant of decompression as the sample passes from the high pressure environment inside the vessel to atmospheric pressure on the outside. A rapid flow rate is not required in order to attain maximum disruption since disruption occurs on an individual cell basis and is independent of the rate at which cells are released from the vessel. At all flow rates, this process is assisted by the vigorous agitation which develops as the homogenate flows through the valve.

Any suitable container can be used to collect the sample as it is released from the delivery tube. A side arm suction flask works well for this purpose. Simply insert the delivery tube into the flask and close the neck with a cotton wad or other stopper but leave the side arm open to release the excess nitrogen. To prevent splattering in the receiver and freezing in the discharge tube, it is well to close the discharge valve after the bulk of the sample has been recovered, then release the remaining nitrogen through the inlet valve. The inlet valve can also be used to release the pressure should it become necessary to abort a test after the vessel has been pressurized. All pressure within the vessel must be released before attempting to open the vessel.

Some investigators working with small samples of very fragile cells have found that such materials can be treated satisfactorily by decompression within the vessel without running the sample through the discharge valve. In these special cases, the pressurized sample is allowed to remain in the vessel while the excess nitrogen is released through the inlet valve. After the pressure has been reduced to atmospheric, the vessel can be opened to recover the sample. Although this procedure is effective for certain types of cells, in most cases it is best to release the sample through the discharge valve to utilize the instantaneous decompression which occurs as cells pass through the valve.

Applications and Techniques

Mammalian Cells

Hunter and Commerford[1] published a paper in 1961 which has become a basic "cookbook" for the disruption of mammalian tissue by the nitrogen decompression method. Although most of the work reported by these authors was done with rat tissue, they also treated spleen, white cells, lymph nodes, tumors, thymus and other tissues to establish the general applicability of the method. Their results clearly demonstrated that cells can be disrupted by this method with minimum physical and chemical damage to the components.

H & C obtained complete disruption at pressures of 1300 psi and above, while pressures below 700 psi left whole cells and clumps of cells in the homogenate. At pressures between 800 and 1000 psi, cell-free homogenates were produced with nuclei intact. A hand press was used to pre-mince tissues prior to treatment in the vessel. The condition of the nuclei was found to be dependent upon the composition of suspending buffer solution. Good results were obtained using isotonic solutions while nuclei swelling and rupture were observed in cells suspended in very dilute solutions. This was attributed to osmotic swelling which H & C found could be controlled by adding inorganic salts such as sodium chloride or organic solutes such as sucrose or glycerol. The nuclei were extremely fragile when the suspending medium contained no calcium, but the presence of as little as 0.0002M calcium chloride was found to stabilize the nuclei. Magnesium acetate is also useful for this purpose.

To determine the extent of damage to labile cells, H & C studied Deoxyribonucleoprotein, DNP, because of its susceptibility to chemical and physical stress, obtaining recoveries of over 90% DNP from the nuclear fraction with excellent preservation of the material. They also compared the enzyme activities of mitochondrial suspensions prepared by the nitrogen decompression method with suspensions produced in a Potter-Elvehjem homogenizer. No differences in enzyme activities were detected.

Dowben, Gaffey and Lynch[2] used the nitrogen decompression technique to prepare polyribosomes from L Cells, fibroblasts, human fetal cells from amniotic fluid, rat livers and muscle from chick embryos. Using 600 psi pressure they obtained better than 99.9% rupture and recovered more than 95% of the nuclei intact. Polysome yield was two to three times greater than when the cells were homogenized in a Dounce tissue grinder. In addition, they had better defined and more reproducible profiles. Significantly greater activities, as measured by amino acid incorporation, were also reported.

Short, Maines and Davis[4] compared the nitrogen decompression method with the Potter-Elvehjem types of Teflon pestle and glass tube homogenizers for preparing microsomal fractions for drug metabolism studies. The decompression method consistently produced over twice as much microsomal protein per gram of tissue as the pestle and tube fractionation. Enzyme activity per milligram of microsomal protein was found to be essentially the same for both methods, but it must be remembered that nitrogen decompression yielded over twice as much microsomal protein per gram of starting material.

Under microscopic examination the homogenates produced by the decompression method were found to be cell-free, while numerous cell clumps were observed in the pestle and tube

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homogenate. Electron microscopy of the microsomal pellets showed the particles to be smaller and more uniform in size for the decompression method. In summary, these authors stated that the nitrogen decompression method was more efficient and probably less variable than the Teflon pestle and glass tube methods.

Comparison with pestle and tube methods. In an application at the Veterans Administration Research Hospital in Chicago, a homogenate that had required eight hours to produce with the pestle and tube was prepared in fifteen minutes with a cell disruption vessel. In another laboratory, up to 12 kilograms of brain per day are being homogenized with a cell disruption vessel.

Wallach and his associates[5] have used the nitrogen decompression method to obtain complete cell fractionation with minimum nuclear damage. Working with Ehrlich Ascites Carcinoma Cells using a 0.0002M magnesium acetate buffer, they have studied the cellular distribution of phospholipides. Wallach has published many other papers in which the decompression technique has been used to prepare cell membranes.

Vaccine Preparation

A number of commercial laboratories have found that the nitrogen decompression technique is extremely effective for releasing virus from fertilized eggs. This method can be scaled up for commercial production using larger disruption vessels which are offered for this purpose by Parr.

Bacterial Cells

While some notable successes have been achieved using this technique with bacterial cells, the disappointments certainly outweigh the successes. Remember the technique is a "gentle method" which depends upon disolving a sufficient quantity of nitrogen into the cell to cause the rupture as the pressure is released. Bacterial cells have small volumes of liquid and tough cell walls; not a combination readily receptive to the nitrogen decompression technique.

Fraser[7] **in 1951** published some of the earliest studies on nitrogen decompression and its effect on E Coli. Fraser's work was limited because his vessel was restricted to 900 psi operating pressure. Nevertheless, he was able to obtain 75% rupture in one pass and over 90% rupture in two successive passes using E Coli harvested during the log growth phase. Results with other bacteria and organisms with tough cell walls have been mixed.

There are several ways in which bacterial cells with tough walls can be treated to facilitate disruption by the nitrogen decompression method. These include: (1) harvesting the cells during an early growth phase before the full wall is developed; (2) growing the cells in the presence of an agent which will inhibit the formation of the cell wall; (3) using a lysozyme to weaken the wall prior to processing; or (4) using a mechanical pretreatment to weaken the cell walls before applying the nitrogen decompression method. Although these techniques have been applied successfully to many bacteria with heavy cell walls, they are not equally effective for yeast, fungus, spores and similar cells with very heavy or hard walls. Vigorous mechanical methods are generally required to break down the cell structure in these hard-walled materials since they generally do not respond well to treatment by the nitrogen decompression method.

Plant Cells

Loewus and Loewus[10] have published a number of papers in which they describe the application of nitrogen disruption procedures to plant cells and to tissue cultured plant cells. They also report considerable success in breaking diatoms by this method.

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The PARR Warranty

Parr Instrument Company (Parr) cell disruption vessels, and associated products are designed and manufactured only for use by or under the direct supervision of trained professionals in accordance with specifications and instructions for use supplied with the products. For that reason, Parr sells only to professional users or distributors to such users. Parr produces precision equipment and associated products which are **not intended for general commercial use**.

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- Will function as described in corresponding Parr sales bulletins, or for specifically engineered assemblies, as stated in the sales proposal and purchase agreement.
- Will remain free from defects in materials and workmanship for one year from date of delivery of the product to the original purchaser/user. **Note** that there is no guarantee of a service life of one year after delivery.

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As to the original purchaser/user and to the distributors to such users, Parr limits its liability for claims other than personal injury as follows:

- **Replacement or repair**. With respect to express warranties herein, Parr's only obligation is to replace or repair any parts, assemblies or products not conforming to the warranties provided herein.
- **Disclaimer of consequential damages**. In no event shall Parr be liable for consequential commercial damages, including but not limited to: damages for loss of use, damages for lost profits, and damages for resulting harm to property other than the Parr product and its component parts.

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