INSTRUCTION MANUAL

VirTis
VIRSONIC 100

6/00

Produced by Joan Miller

TABLE OF CONTENTS

SAFETY PRECAUTIONS

Read ALL Instructions Before Installing or Using Equipment

VIRSONIC 100
Ultrasonic Homogenizer
Instruction Manual

Your new VIRSONIC 100 Ultrasonic Homogenizer has been designed and tested to assure maximum operator safety. However, no design can completely protect against improper usage which may lead to bodily injury and/or property damage. For total safety and equipment protection, read the instruction manual carefully before attempting to operate this equipment. Observe the following **WARNINGS:**

- High voltage is present in the generator (power supply), convertor and high frequency cable. Do NOT attempt to remove the generator cover or convertor case. Do not touch open connection with power engaged.
- Do NOT operate generator with convertor disconnected
- The VIRSONIC 100 Ultrasonic Homogenizer must be properly grounded with a 3-prong plug. Test electrical outlet for proper grounding before plugging in unit.
- Install the VIRSONIC 100 in an area free from excessive dust, dirt, explosive and corrosive fumes and from extreme temperature and humidity.
- NEVER touch a vibrating horn or probe.
- NEVER immerse the convertor in liquids of any kind.
- Refer to the enclosed safety reminder.

S A F E T Y R E M I N D E R

VirTis has supplied the laboratory and research industry with VIRSONIC 100 to handle a wide variety of applications. As with any product of this kind, some applications are more severe than others, resulting in our equipment being subjected to harsh environments and aggressive handling.

Safety precautions regarding the operation and handling of high voltage equipment is prominently indicated in the instruction manual. This letter serves as a safety reminder to the operators of the VIRSONIC 100 to visually and physically inspect the unit to insure optimum and safe performance.

This inspection should be scheduled as a routine maintenance procedure, and done with the VIRSONIC 100 in the OFF position with the unit unplugged from the AC power source.

Long exposure to acids or caustics results in corroding metal parts or components. Check the rear of the generator, convertor, and cables for any signs of rust or discoloration. If discoloration is found, move the VIRSONIC 100 away from the source of the contaminant.

Examine the condition of the high voltage cable which attaches the convertor to the power supply. Inspect the wire insulation for damage, such as burning from hot plate contact, or wear or breakage from extended use or rough handling.

Inspect the cable connectors by gently pulling on the wire while holding the body of the connector. The cable connector and rubber boot protector at the end of the cable should be tight to the wire, with no movement possible and no cracks or frayed ends visible. Do not subject the cable ends to severe bending loads while performing these tests. Return the convertor assembly immediately if your cable does not pass the above inspection.

Should the convertor/cable assembly be subjected to misuse, such as dropping or a severe pulling force on the wire itself, the cable must be inspected as above.

Should the VIRSONIC 100 stop functioning or if it cannot be tuned, shut the unit off and inspect cable as above BEFORE any other action is taken.

DO NOT USE A CABLE WITH BROKEN END CONNECTIONS, EXPOSED WIRES OR FRAYED INSULATION. HIGH VOLTAGE IS PRESENT IN CABLE AND MAY POSE A SHOCK HAZARD. DO NOT TOUCH CONVERTOR ASSEMBLY UNTIL POWER SWITCH OF GENERATOR IS IN OFF POSITION AND UNIT IS UNPLUGGED.

If the operator is in doubt as to the condition of the unit, call your local VirTis customer service representative for prompt attention.

In general use, the cable assembly should not be used to carry the convertor or pull it toward the user. Make certain the cable always has slack and is never tensioned. Move the generator or convertor assembly closer to one another to accomplish this. If this is not possible, contact VirTis customer service to obtain a longer assembly.

SPECIFICATIONS

Generator:

Dimensions: 33cm W x 19cm D x 17cm H (13" x 7.5" x 6.7")
Weight: 4.2 kg (9.2 lb.)
Input Voltage: 115 VAC nom. (switchable to 220 VAC nom.) 50/60 hz
Full Load Current: 1.65 amps at 115v/ 0.8 amps at 220v
Fuse Rating and Type: 3 amp/1.5 amp Fast acting
Voltage Tolerance: +/- 10% nominal voltage
Output Voltage/Frequency: 950 Vrms (maximum) 22.5 kc (nom)

Convertor:

Dimensions: 17cm L x 3cm Dia. (6.7" L. x 1.18" Dia.) without probes
Weight: .2 kg (.5 lb.)
Materials: Aluminum case and Front Driver

Environmental:

Pollution Degree 1
Temperature Limits: 50° F - 110° F (10° C - 43° C)
Barometric Pressure: Unlimited (Note: In high vacuum areas, additional cooling provisions may be needed. Contact factory)

Accessories:

Use only accessories and probes listed in the catalog by the manufacturer as suitable for use with this appliance. Do not attempt to fabricate ultrasonic tooling or accessories unless approval has been obtained from the manufacturer in advance.

I. INSTALLATION

WARNING !!

- **High voltage is present in the generator (power supply), convertor and high frequency cable. Do Not attempt to remove the generator cover or convertor case**.

- **Do Not touch any open cable connections on the unit while the power is turned on.**

A. Power Requirements

The generator requires a single phase, grounded, three-wire, 115V or 220V, 50/60 Hz source, unless otherwise fitted, and has a 3 ampere fuse for 115V service or a 1½ ampere fuse for 220V service. There is a select switch on the rear panel of the generator to set voltage for 115V (92 - 140 RMS) or 220V (200 - 240VRMS) service.

CAUTION: Do Not operate a unit set for 115V service on a 220V line or operate a unit set for 220V service on a 115V line.

WARNING: The electrical line cord is equipped with a 3-prong grounding plug. Do NOT remove the grounding prong under any circumstances. The plug must be plugged into a mating 3-prong grounded outlet.

NOTE: ONLY use IEC approved fuses. Do NOT use "slow blow" fuses or fuses rated above the amperage noted.

Cleaning Instructions

The generator and convertor may be cleaned using Windex™ or a similar acid-free cleaning solution, and an anti-static cleaning cloth. Probes can be cleaned with isopropyl alcohol and sterilized in an autoclave (probes only).

B. Inspection

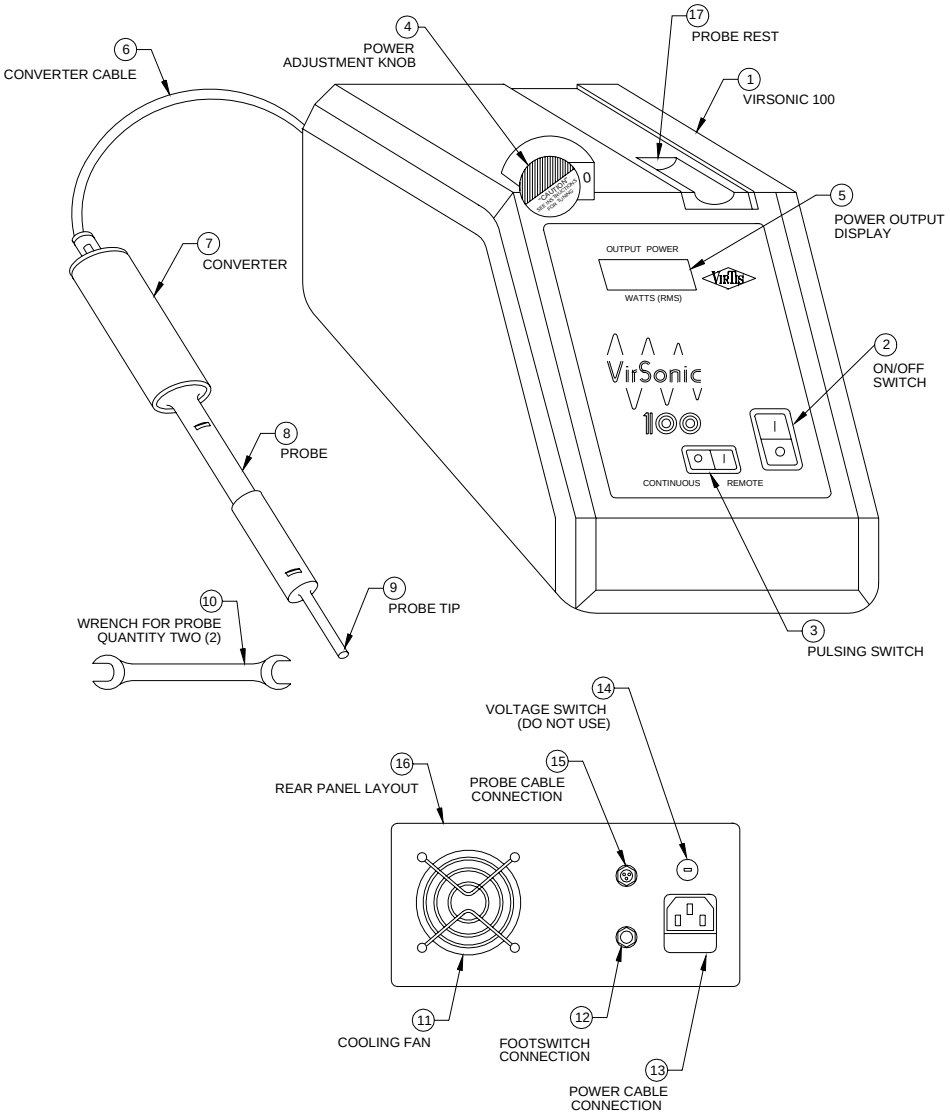
Your new VIRSONIC 100 was thoroughly inspected, tested and carefully packed before leaving the factory. Prior to unpacking, carefully inspect the shipping carton for any evidence of damage. Claims for loss or damage sustained in transit, must be made with the shipping company.

Unpack the unit from its shipping carton and check the contents against the packing list. Before disposing of the packing material, check it carefully for small items. Report any missing components to VirTis immediately.

Visually inspect all external controls, indicators, and surfaces to detect any damage in transit. If damage has occurred, contact your carrier within 48 hours of delivery date. DO NOT OPERATE DAMAGED EQUIPMENT. Retain all packing material for future shipment.

C. Placement of Equipment

A built in fan positioned at the back of the unit draws in cool air from the room to provide radiant and convectional cooling of the internal components. Therefore, **do not block the fan inlet.** Position the generator so that air flows freely around the entire case.


6
CONVERTER CABLE
4
POWER
ADJUSTMENT KNOB
17
PROBE REST
1
VIRSONIC 100
5
POWER OUTPUT
DISPLAY
7
CONVERTER
OUTPUT POWER
WATTS (RMS)
VirTis
2
ON/OFF
SWITCH
VirSonic
100
8
PROBE
CONTINUOUS
REMOTE
9
PROBE TIP
10
WRENCH FOR PROBE
QUANTITY TWO (2)
3
PULSING SWITCH
14
VOLTAGE SWITCH
(DO NOT USE)
16
REAR PANEL LAYOUT
15
PROBE CABLE
CONNECTION
11
COOLING FAN
12
FOOTSWITCH
CONNECTION
13
POWER CABLE
CONNECTION

II. OPERATION

A. Principle of Ultrasonics

The generator (power supply) converts conventional 50/60 Hz AC line power to 22.5 kHz electrical energy which is fed to the convertor where it is transformed to mechanical vibration. The heart of the convertor is a lead zirconate titanate electrostrictive (piezoelectric) crystal which, when subjected to an alternating voltage, expands and contracts. The convertor vibrates in the longitudinal direction and transmits this motion to the MICROPROBE which is immersed in the biological or liquid process solution. Cavitation results, in which microscopic vapor bubbles are formed momentarily and implode, causing powerful shock waves to radiate throughout the sample from the tip face. MICROPROBES amplify the longitudinal vibration of the convertor; higher amplification (or gain) results in more intense cavitational action and greater disruption.

The convertor is tuned to vibrate at a fixed frequency of 22.5 kHz. MICROPROBES are resonant bodies, also tuned to vibrate at 22.5 kHz; any change in mass or geometry can disturb the resonant frequency and cause failure or damage to the convertor or generator.

B. Description of Major Components

1. GENERATOR (also known as the "power source") - includes the operating controls and power indicator, an On/Off switch, and separable three-wire grounded line cord with integral U.S. plug or Europlug, fuse, and high frequency cable connector.

2. CONVERTOR (also known as the "transducer") - includes the transducer crystals, housing, and front driver (first stage of acoustic amplification) with ¼-20 threaded hole for the MICROPROBE.

3. MICROPROBE (also known as the "probe" or "tip") - resonant body with ¼-20 stud, serving as a second stage of acoustic amplification.

C. Description of Operating Controls and Power Indicator

The front panel contains the Wattmeter with Digital LCD Display for monitoring power output, the Power Control Knob, Continuous/Remote Operation Switch, Convertor Rest and On/Off Switch. The **Wattmeter** measures the power (in watts) delivered to the convertor and probe, while the **Digital LCD Display** provides a continuous read-out of this value. The **Continuous/Remote Operation Switch** provides continuous operation or manual pulsing (utilize the thumb switch). **The Power Control Knob** provides continuous adjustment of probe intensity with graduations from 1 to 20. The **Convertor Rest** holds the convertor and probe when not in use,

and the **On/Off Switch** contains a power light indicator. The back panel contains the line fuse and footswitch jack for use with **optional plug-in timer** or **On/Off Footswitch** (see page 10).

D. Preparation for Use

1. The VIRSONIC 100 is easy to set-up. Simply attach one end of the convertor cable to the back of the generator. Plug one end of the power cord into the back of the unit and the other end into a three-pronged (grounded) wall outlet. Then make sure that the MICROPROBE is securely attached to the convertor. The unit is now ready to use.

2. To verify tightness of the MICROPROBE, use the open end wrenches supplied. To install a new probe, first screw the probe into the convertor finger tight only. Then tighten the probe using the wrenches provided by placing the wrenches in the slots of the probe and the (black) front driver.

3. Instead of hand-holding the convertor, a simple three-prong lab clamp and lab stand may be used to hold the convertor and sample tube. Secure the clamp only to the large (1¼ or 32mm) diameter of the convertor. The movement of the MICROPROBE will be restricted if the clamp is placed on the probe or on the (black) front driver.

4. **CAUTIONS: -Never Touch the tip of a live probe to your hand or skin.**

5. **-Always allow the unit to reach room temperature before operating.**

6. **-Do not operate the generator without the convertor attached.**

7. **-Do not let the probe vibrate in air for more than just a few seconds.**

8. **-Avoid touching the activated probe to the sides or bottom of the sample container, and do not place it down on the work surface**

9. **-Do not use the On/Off switch for pulsing, only use the optional plug-in footswitch to manually pulse the probe.**

E. Operation

1. The VIRSONIC 100 does not require manual tuning. It has an automatic tuning feature and is fully self-tuning across a wide band of load conditions. To operate, follow these instructions:

-Simply hold the body of the convertor.
-Turn the power control knob to zero.
-Turn the power switch on.

-Allow digital reading to stabilize at _ 0.0.

-Place the probe into your sample with the tip at mid-depth.

Continuous Operation

-Switch front panel switch to continuous operation
-Adjust power setting to the appropriate intensity level for your sample

Pulsed Operation

-Switch to remote on front panel switch
-Pulse as desired using transducer pulse button switch with appropriate intensity power setting

Footswitch Operation

-Switch front panel switch to continuous
-Insert footswitch plug into jack on rear of the unit
-Set desired power intensity level
-Pulse as desired using footswitch

1. The sides and end of the tip must never be allowed to come in direct contact with the container or hard surface. The stress resulting at the point of contact could cause fracture of the MICROPROBE or of the glass container.

2. Immerse the tip of MICROPROBE at least 1 to 1½ deep into the solution (without touching the bottom). Immersion depth can be less for larger MICROPROBE tips and may have to be more for smaller MICROPROBE tips used at high output control settings.

3. Lower the tip into the solution to avoid aerosoling and foaming. Aerosoling and foaming generally occur when the tip is not immersed far enough into the solution, or if too high of a power setting is used. By lowering the tip in the solution, decreasing power, and reducing solution temperature will normally prevent aerosoling and foaming. In severe cases, use a narrow test tube and plastic film over the vessel. Push the tip through the film to sonicate. Be sure to clean the tip before sonicating another sample, since protein released from cell material acts like a wetting agent and tends to promote foaming. Once foaming occurs, reduce power below the cavitation level before proceeding. If the foam persists, the sample may have to be discarded.

4. The highest intensity energy is concentrated directly under the tip and dissipates within ¼" (6mm) from the tip. Liquids and suspended solid to be processed must circulate freely in this zone. Whole tissue should first be finely divided or homogenized roughly in a mechanical device, such as a laboratory blender or rotary homogenizer, before being sonicated. Small pieces of tissue can be sonicated whole if trapped directly under the probe tip.

5. **Operation with optional Plug-in Timer**

6. The timer allows the probe to be activated for a specific period of time between 1 to 15 minutes. At the end of the timed cycle the probe will be inactive indefinitely (the power to the unit remains on, however).

For timed operation:

1. Turn the power control knob to zero.
2. Turn the power switch on.
3. Allow digital reading to stabilize at ~ 0.0.
4. Place the probe into your sample at mid-depth.
5. Insert Timer plug into the footswitch socket on rear panel of generator.
6. Adjust the power setting to the appropriate intensity level for your sample.
7. Set the desired time on the timer. Once timer is set the probe will be activated. Unit will shut off at end of timed cycle. Repeat as necessary to process all samples.

7. **Operation with optional Plug-in Footswitch**

The footswitch allows the user to manually activate the probe. The user may switch the probe on or off at their own discretion by depressing the footswitch. The footswitch is commonly used for "pulsed" sonication or for "hands-free" operation, allowing the operator to manipulate the sample with their hands.

For foot switch operation:

1. Turn the power control knob to zero.
2. Turn the power switch on.
3. Allow digital reading to stabilize at ~ 0.0.
4. Place the probe into your sample at mid-depth.
5. Insert Footswitch plug into the footswitch jack on rear panel of generator.
6. Adjust the power setting to the appropriate intensity level for your sample.
7. Depress footswitch to activate output.
8. Hold footswitch down for desired period of operation.
9. Release footswitch to de-activate output. Repeat as necessary to process all samples.

CAUTION: If Footswitch or Timer plug is removed from jack at rear of unit, then the unit will remain on indefinitely. In this event turn main power switch off to disengage unit.

F. Care of MICROPROBE Tips

Proper care of MICROPROBE tips is essential for good performance and long service life. Tightness of the probe, cleanliness of the mating surfaces and condition of the probe tip are all very important to overall performance. The probe tip is continuously subjected to intense shock waves which cause cavitational erosion of the probe's tip. Keeping the tip face smooth and polished will significantly improve sonication efficiency and increase the weekly:

-Check the tightness of the probe periodically with the open-end wrenches provided.

-Keep the stud threads and the mating surfaces between the probe and the (black) front driver clean and dry.

-Check the probe tip for signs of cavitational erosion and pitting.

-Polish the probe tip with a very fine emery cloth or sandpaper (#600) to remove any surface scratches or pitting.

-Do not file or grind the probe tips as this could bend or break them.

-Replace probes periodically, especially if they no longer tune properly, are badly pitted or eroded, or if they are bent or cracked.

III. REPAIR AND REPLACEMENT

A. Return of Equipment

All requests for repairs and replacement parts should be directed to the Instrument Service Department of VirTis at (800) 431-8232. Always provide Model Number and Serial Number of both the generator and convertor with all requests for parts or service. In order to receive prompt attention, contact the Service Department and obtain an RGA (Return Goods Authorization) Number before returning any equipment. Always return the generator, convertor and probe. Include a note with the unit stating the Model Number and Serial Numbers of both the generator and convertor, the RGA number and a brief explanation of the problem with the unit. If possible include:

-A Purchase Order Number.
-Bill To and Ship To address.
-The return method of shipment (usually we ship UPS Ground, prepaid and add).
-**International Returns** - Ship back to us via Air Freight. Freight Charges Prepaid by you and Free Domicile.

C A U T I O N

When using loose packing materials, such as foam pellets, shredded paper, or excelsior, be sure to wrap the generator and convertor separately in plastic bags or plastic wrap. Remove MICROTIP probes and pack separately in same outer carton.

APPLICATION LIST

Acetobacter Suboxydans - completely disrupted in a few seconds.

Actinomyces - 3 minutes disruption produced excellent disruption with 50% protein released and excellent enzyme activity.

Actinomycin D - suspended or dissolved in 3 minutes.

Aerobacter Aerogenes - excellent breakage with better enzyme release than any other method. A low power setting can release sulfatase activity into the supernate with no obvious disruption of the majority of cells.

Algae Scenedesmus - 10ml concentrated solution completely disrupted in 1 minute.

Alkaloids - total amount as well as speed of extraction is greater using ultrasonic disruption. 30 seconds processing of ipecac root yielded more alkaloid than Soxhlet extraction in 5 hours.

Antibioticus - monocellular elements from surface-grown colonies obtained in 1 minute, 50% disruption in 2 minutes, completely disrupted in 5 minutes.

Antigen - ultrasonic processing is extensively used to produce antigens and vaccines, either to increase yield or expose otherwise unobtainable sites.

Antigen/Antibody Complexes - broken apart using ultrasonic disruption.

Aorta - 1 gm completely disintegrated in 2 minutes.

Aphanomyces - afterblending, completely disrupted in 3 minutes.

Arthrobacter Tumescens - 10 gm in 40 ml disrupted in 5 minutes for 0 coumaric reductose.

Ascaris Eggs - concentrated solution. 8 ml. complete breakage in 3 minutes.

Asperigillus - completely disrupted in 4 minutes.

Aurefaciens - monocellular elements from surface-grown colonies obtained in 1 minute. 50% disruption in 2 minutes. completely disrupted in 5 minutes.

Azotobacter Vinelandii - 15ml buffered solution. 200 mg. wet weight per ml. completely disrupted in 2 minutes.

Bacillus - stereothermophulus (Thermophillic spore form) 98% disruption of 70 ml of 40% suspension in 15 minutes.

Bacteroides Symbiosis - 1 phosphofructokinase. a soluble enzyme has been isolated from this anaerobe by ultrasonic treatment. A 25 ml suspension was disrupted for 10 minutes and centrifuged at 36,000 Xg for 10 minutes.

B. Anthracis - 80% disruption in 4 minutes. 10ml of eryisipelothrix rhusipathiae was completely disrupted in 10 minutes.

B. Cereus Veg Cells - completely disrupted in a few seconds.

B. Cereus Spores - disruption in 6 ml 13 minutes.

B. Megaterium Spores - concentrated 6 ml solution. Complete breakage in 15 minutes.

B. Stereothermophilis Spores - completely disrupted in 2 minutes.

B. Subtilis - disruption of 5 gm wet weight, 15 ml buffer, in 5 minutes.

B. Subtilis Veg Cells - heavy suspension clears in 1 minute.

Baker's Yeast (Saccharomyces Cerevisiae) - 9gm pressed yeast in 18 ml buffer, completely disrupted in 8 minutes. Protein release, 52 mg/ml from an aged sample.

Blastomyces Dermatitidis - 95% disruption in 3 minutes.

Blood Cells - red and white cells can be disrupted in a few seconds.

Boll Weevil Tissue - complete homogenization in a few seconds.

Bone - compact bone can be ultrasonically processed for microscopic sections in minutes as opposed to several days or even a week of other methods. Bone specimens treated in this way yielded large numbers of intact cells with little distortion. Malignant criteria were easily recognized. Tumor types studied were osteosarcoma, chondrosarcoma, liposarcoma, chordoma, metastatic bronchogenic squamous and benign giant. Bone can be decalcified without injury to the cells in a short time, processed for microscopic sections, and diagnosed. Other methods require extensive treatment time.

Brain Stem and Adrenal Gland - dispersion of 10 mg samples in 10 ml fluid, normally difficult without substantial loss of material. Suspension analyzed for nucleotides.
Brain Tissue - completely disrupted instantly.
Brevi Bacterium - 25 ml completely disrupted in 20 seconds.
Brevi Bacterium Acetylicum - approximately 3 minutes to disrupt large samples and measure TCA enzyme activity.
Brine Shrimp - completely disrupted in 1 minute.
Brucella Abortes - easily separated from leukocytes. At least 9 antigens extracted.
Bull Sperm - contractile protein more easily extractable from tails after disruption.

C. Butyricum - vegetative cells easily disrupted.

C. Cylinrosporum - vegetative cells easily disrupted.

C. Kluyveri - vegetative cells easily disrupted.

C. Pasteurianum - 3 minutes disruption for hydrogens reducing Ferredoxin with H_2.
Calcium - mouse Ehrlich ascites tumor cells were disrupted for 1 minute to determine the amount of bound calcium present. Cells were labeled with calcium 45.

Candida Albicans Spores - 95% disruption in 35 minutes. 15 ml solution, 1/2 gm dry weight.
Carbon Black - excellent small particle suspension and deagglomeration. **Caryophanon Latum -** disruption yields glucosamine, muramic acid, alanine, glutamic acid and lysine.
Catecholamine - can be extracted from heart muscle through disruption.
Cellumonas Biazotea - disruption obtained with retention of malate dehydrogenase activity.
Chemical (and Physical) Reactions - accelerated by ultrasonic disruption, as are enzymatic processes.

Chicken Sperm - 30 ml completely disrupted in 2 minutes.

Chlorella - 10 ml completely disrupted in 3 minutes.
Chloroplasts - disrupted in a few seconds.
Cholesterol - apparent permanent suspension in 1 minute in water.

Chromatography - prior ultrasonic treatment of absorbant in any convenient solvent for a few seconds eliminates aggregates and results in a uniform, easily packed column.
Clostridium - completely disrupts all types.
Coagulase-Globulin - disruption before precipitation yields much more enzyme.
Collagen - an excellent fragmentation.
Colletotrichum Capsici Spores - 5 ml with 6 million spores/ml, completely disrupted in 4 minutes.
Corticosteriod - particle size can be reduced to approximately 5 microns. Large volumes can be treated at the rate of approximately 30 ml/minute on a continuous flow basis.
Corynebacterium - completely disrupted in 4 minutes with 50% protein release and excellent enzyme activity.
Cryptococus Laurentii - completely disrupted in 7 minutes with good protein release and enzyme activity.
Cryptostroma Corticale (Maple Bark Spores) - concentrated 6cc solution. completely disrupted in 14 minutes.
Crystal Reduction - large crystals of an organic compound suspended in isopropanol can be reduced in diameter by 10 to 40%.
Cyanidium Caldarium - concentrated 5 ml solution completely disrupted in 6 minutes.

Decalcify - bone may be decalcified without injury to the cells, processed for microscopic sections and diagnosed in a short time - as opposed to several days or even a week by other methods.
Dental Plaques - 5 ml solution, concentration 1 to 10,000 low power setting. 53,500,000 organisms per ml were obtained in 45 seconds.
Desulfovibrio Vulgaris - in less than 30 seconds TCA enzymes were released.
Diplococcus - completely disrupted in 5 minutes.
DNA - breaks chains on low power instantly. Controlled degradation may be obtained.
Dyes - excellent rapid dispersion and homogenization.

E. Coli - 2 gm wet weight in 10 ml solution, completely disrupted in 40 seconds.
Egg Whites - can be reduced to homogeneous. pipettable solution in 15 seconds on low power.
Ehrlich Ascites - completely disrupted in a few seconds.
Electron Microscopy - used to clean apertures.
Embryonic Duodenum - a 1 ml sample is easily homogenized in 15 seconds with a microtip.

Emulsions - 10 ml of most light mixtures become semi-permanent emulsions in about 1 minute without emulsifiers; average particle size is usually well under 1 micron. Sterile emulsions can be prepared by ultrasonic treatment for feeding to germ-free animals.

Enterococcus - excellent disruption.

Erwina Cartovara - completely disrupted in 1-2 minutes depending on cell concentration.

Erythrocytes - easily disrupted in a few seconds.

Euglena Gracilis - completely disrupted in a few seconds to isolate chloroplasts.

Eugoena - 90% disruption in 8 minutes with pigment released. Completely disrupted in 12 minutes.

Extraction - excellent for oils, fats, and lipids, alkaloids.

Fasciola Hepatica - completely disrupted in less than 1 minute.

Fax Extraction - fat may be emulsified without injuring tissue with proper power selection. Lipid layer can be stripped from spores and mycrobacteria.

Fibrin - complete suspension 1/8gm in 30 minutes.

Fish Gill - 20 mg completely disrupted in 30 seconds.

Fish Tissue - tissue homogenization for extractions, excellent particle size reduction, 8 minutes per 10 gm.

Fluorocarbons - extended treatment time will break down particle size to well under 1 micron and gives a fine homogenate.

Fossils - low power will clean debris from delicate fossils without injury. Micro fossils such as pollen can be separated from rocks to help identify the geological age of the strata. Removal of the rock matrix.

Fuel Oil and Water - permanent emulsions without wetting agents can be formed on continuous flow basis.

Gamma Globulin - ultrasonic disruption is used to solubilize protein as one of the steps in the biosynthesis of gamma globulin from rabbit spleen.

Gangliosides - immunochemical and structure studies were aided by an ultrasonic treatment as one step during the procedure.

Gastric Mucosa - placing scrapings into a test tube immersed in Cup Horn permitted these cells to be separated and not broken.

Germ Free - ultrasonic disruption is a good method for preparing sterile emulsions fed to germ free animals.

Graphite Molybdenum Disulfide - an excellent dispersion of this lubricant was made in silicate binder.

Guanine - produced colloidal suspension in 1 minute.

Gymnodinium - completely disrupted 10 ml solution in 6 minutes.

Heart Muscle - completely disrupted 1 gm in 6 minutes.

HeLa Cells - disrupted to free virus in a few seconds without injury.

Hemophilus Pertussis - prepared an immunological compound.

Herpes Virus - may be quickly released without injury.

Histoplasma Capsulatum - disruption for 7 minutes completely ruptured cells prepared by formalin fixation. Good enzyme activity was obtained.

Human Serum Proteins - ultrasonic disruption caused a reproducible change in the elctrophoretic behavior of normal human serum consisting of an increase in material of migrating in the x and b globulin zones with a reduction in the albumin and y globulin fractions.

Hydrocortisone - smaller crystals were produced by disruption.

Hydrophilic Vegetable Gums - dispersed and solubilized hydrophilic vegetable gums in water. made dispersions of added particulate matter.

Intracellular Membrane - disruption and particle size reduction obtained in 30-60 seconds.

Isoenzymes - selectively activated with respect to time and intensity of treatment.

Kidney - completely disrupted 1 gm in 3 minutes.

Kidney Stones - easily broken in seconds in vitro.

Klebsiella - excellent disruption.

Lactobacillus - completely disrupted 0.5gm in 15 ml in 11 minutes. Excellent release of acetokinase.

L. Arabinosis - completely disrupted to free virus in 2 minutes using high power without injury.

Leuconostoc Mesenteroides - disrupted in 15 minutes using high power.

Leukocyte Lysozme Activity in Myelocytic Leukemia - the cell suspension was processed ultrasonically and samples assayed for lysozyme activity. The

lysozyme concentration of the leukocytes ug/10^6 cells were determined.

Linoleic Acid - made suspension in water in 30 seconds.

Lipid Vesicles - excellent results preparing small, unilamellar phospholipid vesicles with Cup Horn as well as by direct probe contact.

Liver Tissue - 1 gm was homogenized in less than 1 minute.

Lung Tissue - 1 gm was homogenized in 2 minutes.

Lymphacytis - completely disrupted in 15 seconds.

Lymphocyte Nuclei - completely disrupted in 6 minutes.

Lymphography - direct injection lymphography with a modified radiopaque emulsion was obtained by ultrasonic disruption producing lymphatic structure detail.

Lysosomes - released enzymes quickly.

Malaria Protozoa - completely disrupted in seconds.

Maple Bark Spores - completely disrupted in 14 minutes.

Measles - disruption of virus (measles) antigen clumps present in infected cells on low power. Ultrasonic processing increased antigen titer 4-8 fold.

Methanobacillus Omelianskii - completely disrupted for assaying methane. 1 gm cells wet weight/ml of 0.5M, in 2 minutes.

Microbacterium Lacticum - ultrasonic treatment used for malate dehydrogenease extraction.

Micrococci - completely disrupted 13ml solution in 15 minutes.

Micrococcus Lactiliticus - 75 ml of a 20% suspension was disrupted in 15 minutes. A good yield of the enzyme Xanthine dehydrogenase was extracted.

Mineral Rock - excellent for cleaning surfaces between polishing stages.

Mitochondria - separated from cells without injury. Mitochondria themselves can be broken with longer disruption. Inner membrane sub units also isolated.

Muscle Tissue - 1 gm homogenized in 4 minutes; heart muscle 6 minutes.

Mycobacteria - 20 ml growing media completely disrupted in 14 minutes. Clumps broken quickly. Prepared an immunological compound.

Mycoplasma Antibody - a suspension of Campo-W cells treated for 5 minutes gave 12 lines with the sera in a gel diffusion test. The extract was estimated to contain 12.75 mg protein per ml by Blaret reaction.

Myeloma Tumor Cells - completely disrupted in 10 minutes. 30% disruption in 2 minutes.

Myleran - made collodial suspension and dissolved in approximately 1 minute.

Naegleri Gruberi - this free-living soil amoeba was processed to release subcellular infectious material.

N. Crassa - nuclease was isolated and purified from conidial extracts after 5 minute treatment.

Neurospora - 40 ml processed 4 minutes produced more protein than freeze thawing for study of enzymatic synthesis of cystathionine.

Nocardia Ostenodes - disrupted in less than 10 minutes.

Nucleoprotein - extracted from tissue. May be degraded selectively.

Oil and Water Emulsions - permanent. stable emulsions in a few seconds. Particle size reduced to less than 1/2 micron (each case slightly different). Oil-in-water-water-in-oil phases can be obtained in same vessel. Continuous flow process is available.

Oyster Shell - small clean hole can be drilled with microtip in 3 minutes without cracking.

Paracolon - completely disrupted.

Parasites - easily separated from red cells in a few seconds.

Pasteurella Pestis - completely disrupted 20 ml in 30 minutes using high power.

Penicillium - completely disrupted in 3 minutes.

Pesticides - ultrasonic treatment resulted in a 16 fold improvement in the potency of the antigen used with Microcrystalline cellulose as a thin-layer adsorbent for chromatographic separation.

Phosphatidate Phosphohydrolase - the most potent inhibitors for this enzyme were obtained by making five dispersions.

Phospholipid Micelles - produced stable preparations for an indefinite period.

Plant Cells - 30% packed plant cells (W/V) and distilled water (depending on type) can be completely disrupted in 1-15 minutes.

Plant Tissue - 1 gm dried tissue suspended in alcohol was disintegrated in about 5 minutes.

Platelets - completely disrupted depending on size from 20 seconds to 4 minutes.

Pneumococci - preserved in formalin for several years; completely disrupted in 6 minutes.

Polio Virus - excellent disruption.

Powders - are broken down to a small, relatively uniform particle size.
PPLO - completely disrupted in 2 minutes.
Propionobacteria - releases sulphydro groups intact 70 ml of 20% suspension processed 10 minutes.
Propionibacterium Shermanii - 2 minutes for extraction for citrate synthose.
Proteus - excellent disruption.
Pseudomonas Aeruginosa - rapid. completely disrupted.
Pseudomonas Fluorescens - 2gm wet weight in 10 ml completely disrupted in 1 minute.
Pulmonary Cytodiagnosis - the mucus in sputum can be evenly dispersed affording a quick representative sample of cells for cytologic examination. Cells are liberated from the mucus of sputum that had been immersed in 50% alcohol or fixative.

Ragweed Pollen - 15 ml solution completely disrupted in 11 minutes.
Rat Bone - 1/2 gm completely disrupted in 4 minutes.
Rat Liver - completely disrupted in 3 minutes.
Rat Liver Mitochondria - completely disrupted in seconds.
Rat Skin - completely disrupted 1 gm in 4 mnutes.
Red Cells - disruption breaks particle size to 100 Angstroms. Complete disruption in 1 minute. 25 gms/100 ml, saline or plasma, sample treated 15 seconds, 35% disruption. Adenosine triphosphate as shown to be membrane bound by this method.
Reovirus - dissociates cell-bound and aggregated virus. Maximum titer with 4 ml of virus was achieved in 2 minutes.
Retinal Outer Segments - broke particles down to lmost molecular size.
Rhodopseudomonis Palustris - completely disrupted in 4 minutes.
Rhodospirillum Rubrum - completely disrupted in a few seconds.

Rimosus - monocellular elements from surface-grown colonies obtained in 1 minute. Completely disrupted in 5 minutes. 50% disruption in 2 minutes.
RNA - rapid and thorough re-suspension of 9 PCA pellets during extractions.
Rocks - excellent for disaggregation of sedimentary rock. Excellent for cleaning material rock surfaces between polishing stages.

Saccharomyces Cerevisiae (Baker's Yeast) - 9 gm pressed yeast in 18 ml buffer; completely disrupted in 8 minutes. Protein release 52 mg/ml from an aged sample.
Saliva Glands - completely disrupted.
Salmonella - various culture media or phosphate buffered saline disintegrated between 40 and 50% in 10-20 minutes. Disrupting was one step in an improved assay for enzyme thiogalactosize transacetylase.
Salmonella Typhimurium and Enteritidis - bacteria were suspended in 1/300 volume of original culture. processed for 4 minutes and centrifuged for 20 minutes at 20,000 g. Extracts were found to catalyze the synthesis of cytidine diphosphate 3, 6-dideozyhexoses.
Schistosome Mansoni - completely disrupted.
Sedimentary Rock - completely dispersed flocs with the release of all bound silt and clay particles.
Sediments - readily dispersed fine material allowing quick separation of the sand from silt and clay fractions.
Serial Number Restoration - used in crime laboratories to restore obliterated serial numbers.
Serratia Marcescens - complete breakdown in 1 minute for a 12 ml concentrated solution.
Serum - quickly homogenized.
Serum Cholinesterase - different cholinesterase isoenzymes may be activated selectively and inactivated selectively.
S. Faecalis - completely disrupted in 1 minute.
S. Fragilis - 5 minutes yielded excellent release of galactokinase; much more than any other method. Sub cellular particles may be extracted or disrupted.
Shale - excellent disaggregation of all fine grained sedimentary rocks.
Shellfish - by drilling a clean hole with the microtip. various fluids or samples may be withdrawn or injected from living shellfish without destroying the animals.
Shigella - quick disruption.
Skin - completely disrupted 1 gm in 4 minutes. Epidermal homogenates can be extracted that are able to respire and utilize substrate.

Soil - separated solid particles without the use of oxidents, acids or peptizing agents and yielded stable suspensions.
Sperm (Human) - tails are broken instantly. heads are broken in 20 minutes.

Sputum - cancer cells are more easily detected after ultrasonic treatment due to even dispersion of cells and sputum, and complete liberation of the cells from sputum.

Staphylococcus - concentrated solution, 15 ml. 98% disruption in 10 minutes. With 1 gm cells wet weight. to 2 gm water. 54.5 mg/ml of protein was released.

Starch - obtained by extracting from green plant leaf homogenate.

Streptococcus, Group A - 20% suspension in 15 ml solution disrupted in 15 minutes.

Streptomyces - monocellular elements from surface-grown colonies obtained in 1 minute 50% disruption in 2 minutes. Completely disrupted in 5 minutes.

Sub Cellular Particles - may be separated or broken depending upon power selection and length of time.

Sulfanilamide - dispersed in less than 1 minute. Continued processing produced complete disruption.

Sulfapyridine - excellent dispersion in less than 1 minute. Continued processing produced complete disruption.

Synovial Fluid - disruption is an excellent means of reducing fluid viscosity. The ultrasonic method is both simpler and faster than using hyaluronidase.

Tablets - completely disrupted in 2-40 seconds depending on type. Excellent for automatic machines.

Tea - excellent extraction.

Tetrahymena - disrupted in a few seconds. Enzymes which have been monitored include: succinate, lactate, B-hydroxy butyrate, glutamate and DPNH oxidases, DPNH-cytochrome C reductase and ribonuclease. Specify activity of DPNH oxidase was twice that of the best previous experiments.

Thermoactinomyces - disruption of hyphae. Homogenization of protein complex without denaturation.

Thermophile Negative - completely disrupted in 2 minutes.

Thermophilic Bacillus - Isocitrate lyase was extracted from a spore forming bacillus similar to Strearothermophilus. A washed cell paste suspended in phosphate buffer was processed 2 minutes and the supernatant was used for enzyme experiments without further treatment. Extracts could be frozen and stored without loss of activity.

Thiouric Acid - dissolved in a few seconds.

Thymus Cells - completely disrupted in 15 seconds.

Tissue Culture Cells - completely disrupted in a few seconds. To avoid damage to free organelles and to obtain intact lyososomes, use low power at short exposure.

Toxin and Antitoxin - one example of many: Toxin preparation of whole cell lystate (WCL) of the inaba serotype strain 569E of the classic biotype of cholera vibrio were grown on 3% Bactopeptane agar and harvested in distilled water in 18 hours. The unwashed suspensions were solubilized by disruption, clarified by centrifugation and the supernat freeze dried for the titration of cholera toxin in the rabbit ileal loop.

Toxoplasma Gondii - can be separated from white blood cells without injuring.

T. Pyriformis - completely disrupted. 8 enzymes released.

Transplantation Antigens - were extracted from spleen, thymus and lymph nodes.

Trichomonas Foetus - completely disrupted in a few seconds.

Triolein - completely emulsified in 2 minutes.

Trypanosomes - completely disrupted concentrated 10 ml solution in 4 minutes.

Tumor Tissue - disintegrated much faster than normal tissue.

Uterus Muscle - completely disrupted 1/5 gm. 3 cc in solution, 3 minutes for coenzyme Q determination.

Vaccines - numerous advantages such as more antigenic material released than usual and the producing of vaccines not obtainable by classical methods.

Various Bacilli - completely disrupted in 3 minutes.

Vibrio Comma - excellent disruption.

Virus Extraction - excellent for making experimental vaccines. Evidence of breakage of virus/antibody bonds. Virus can be extracted at low power without damage, if broken at high power.

Vitamin E - 30 seconds disruption put material in solution with a resultant permanent suspension.

W138 Virus - Cell free V-2 virus obtained in 30 seconds using 6 ml of Veronal buffer with W138 cells containing V-2 virus.

Yeast - completely disrupted in 3 to 10 minutes depending on type.

Zooplankton - completely disrupted in less than 1 minute.

SPECIFIC APPLICATIONS

Bacterial dispersion in Cup Horn - sonication, with dissolving agents, can dissolve bonds between cells.

Ceramics - fluidization of slurries and casts; suspension and homogenization of fine particles.

Conservation, restoration, and preservation of historical artifacts - such as pottery shards, bones, etc.

Contract Echocardiography Agents - sparging and dispersion of microbubbles in contrast agents.

Degas HPLC Solvents and other liquids for general laboratory use and for quality control (TOC determination) in breweries and distilleries for beers, wines, and spirits, and in soft-drink bottling.

E. Coli - Most commonly used cell in biotechnology for R&D of new pharmaceutical or industrial products. (Plastics, fuel oil cleanups, remedied soil and waste cleanup).

EPA Hazardous Waste Protocol - (Times Beach, Love Canal, etc.) - Homogenization of solid dwaste samples for EPA SW-85, Method 3550, and similar test methods.

Fiber Processing - (cigarette paper, insulators, composites, etc.) - more uniform laydown, denser felting, and deflocculation.

Focused Cleaning - wire dies, blind holes, metal parts, etc.

Microdissection - selective ablation of tissues for electron microscopy.

Plastic Film Dissolution for quality control of electronic insulating materials.

Quality Control of Metal Powder/Pigment/ Paint/Activated Carbon - Deagglomeration and suspension.

Sonochemistry - (primary reference) - generation of new species and acceleration of reactions in laboratory and industry.

Liposomes Preparation - (Cup Horn or Microtip) - Micelles can be formed in cavitation field - medication delivery systems.

DNA and Polysaccharide Cleavage.

Ozonation for remediation in waste treatment.

Restoration of serial numbers on guns and engines in forensics.

Super critical cleaning - high pressure cleaning with liquid CO_2.

Tablet dispersion in the medical and pharmaceutical fields.

Tuberculosis (TB) screening - cup horn for mycobacteria.