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ULTRASONIFICATION OF BIOLOGICAL MATERIALS

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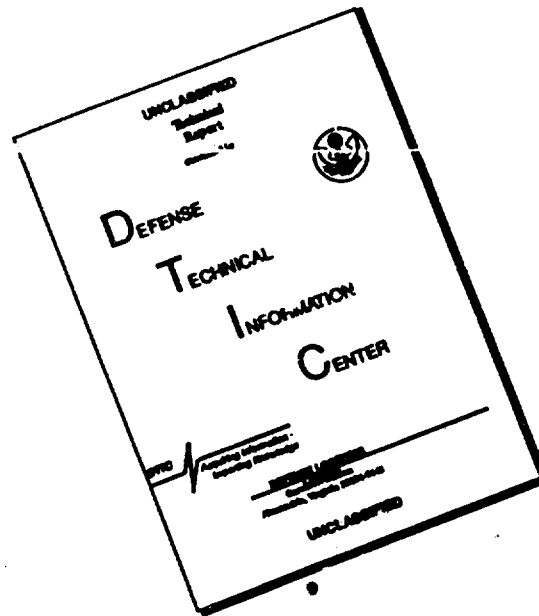
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13. ABSTRACT Ultrasound has potential as a probe of biological structure (1,2). However, difficulties in reproductibility with sonification have occurred in our laboratory. Further, long unprotected exposure to high-frequency sound may be hazardous. We describe here an apparatus which avoids these difficulties.			

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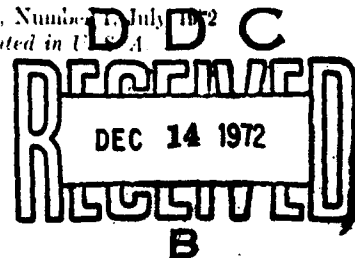
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ANALYTICAL BIOCHEMISTRY 48, 225-232 (1972)

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Ultrasonification of Biological Materials^{1,2}

I. Apparatus

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Ultrasound has potential as a probe of biological structure (1,2). However, difficulties in reproducibility with sonification have occurred in our laboratory. Further, long unprotected exposure to high-frequency sound may be hazardous. We describe here an apparatus which avoids these difficulties.

METHOD

Apparatus

The apparatus for holding the Branson sonifier³ is shown in Fig. 1. It was built by Dale White, Model Shop, National Naval Medical Center.

The upper Plexiglas plate (A) and lower Plexiglas plate (B) hold the reaction tube (O) in position by means of the gum rubber springs (F), which are fastened to the plates by nylon plugs (G).

The reaction unit moves by motion of the stainless-steel guide rods (I). After the position of the probe is fixed by tightening the screws (Q) which lock the guide rods (I), the assembly can be lowered into a temperature bath by loosening the knurled screw (R). The unit can revolve on the shaft (D₂). The brass collar E₁ and its mate (directly opposite to it in Fig. 1) on the two rods (I) fix the vertical position of the stephorn in relation to the reaction vessel (O). E₂ is a similar collar on shaft D₂ which can be placed at position X or Y. It serves to fix the position of the holder C or D₂.

The brass (M) and plastic guides (L) keep the stainless-steel rods

¹ The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

² From the Bureau of Medicine and Surgery, Navy Department, Research Task MR041.06.01.0006AOCK.

³ Branson model W-185-C, Heat Systems Co., Melville, L. I., New York.

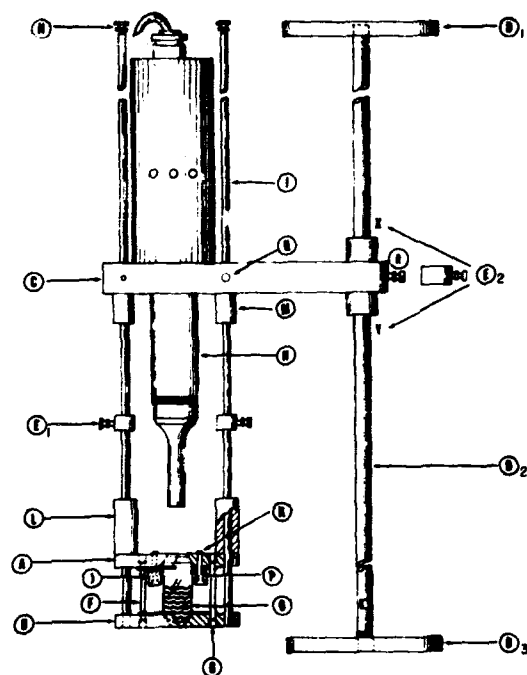


FIG. 1. Sonifier with attached self-aligning holder for reaction vessels (the entire unit is fastened to a floor-to-ceiling support by D_1 and D_3):

- | | |
|--|---|
| (A) Upper Plexiglas plate. | (G) Nylon plugs. |
| (B) Lower Plexiglas plate. | (H) Sonifier. |
| (C) Sonifier holder (aluminum-6061-T6). | (I) Stainless-steel rod. |
| (D) Ceiling-to-floor stand, consisting of 3 parts: D_1, D_2 (aluminum discs), D_3 (stainless-steel bar). | (J) Plexiglas clamp and/or aligners. |
| (E) Positioning brass collars with knurled thumb screws: E_1 for rod I, E_2 for bar D_2 . | (K) Stainless-steel screws (roundhead). |
| (F) Gum rubber springs fastened at either end to nylon plugs. | (L) Lucite aligning cylinder for rod I. |
| | (M) Brass aligning cylinder for rod I. |
| | (N) Knurled thumb screw. |
| | (O) Reaction vessel. |
| | (P) Stainless-steel screw (100° flat). |
| | (Q) Knurled thumb screw. |
| | (R) Knurled thumb screw. |

vertical. The cylinders (M) were pressed into the two openings in the holder (C). The guides (L) were cemented to plate (A).

A cross-section and top view of plate (A) and (B) are given in the left and center sections of Fig. 2. When using the microtip, the plastic adaptor shown in Fig. 2 keeps the sonifier tip centered in the narrow test tubes that are then used. The plastic aligning pieces (see J, Fig. 1) are rotated to lock the adaptor securely into the opening (d).

Support for Converter

The converter and stephorn are placed through the larger opening in the support C until the converter's housing's lower edge is flush with the lower edge of the holder and then locked in with the cad screw (a) (Fig. 2, right section).

Reproducible Positioning of the Reaction Vessel

The slanting walls (Fig. 2) of (A) ensure a reproducible tight fit of the reaction vessel to upper plate (A) and lower plate (B). The height of the tubes that can be used is determined by the stretch length of the rubber springs.

Monitoring Stephorn Output

A General Radio Soundmeter (model 1564-A) and a $35\frac{1}{2}$ in. coaxial cable ($\frac{3}{16}$ in. o.d.) was used to estimate stephorn output (3,4). The coaxial cable is stripped to the center core at one end, forming a loop ($1\frac{1}{16}$ in. in diameter). The loop is placed in a small groove surrounding the opening in plate (A) and is held by a piece of plastic with two screws attaching it to plate (A). When experimental conditions permitted, the more efficient high-frequency microphone was used (3,4).

Soundproof Enclosure of Sonic Source

The sonifier we used produces 120 db of sound, 1 in. from the end of the standard stephorn. Airborne low-frequency ultrasound of 120 db intensity produces changes in auditory and vestibular functions and alters the time of reflex reactions (5,6).

Consequently, a $28\frac{1}{2} \times 25 \times 40$ in. box was built to eliminate the irritating audible noise and the ultrasonic waves from the surrounding area. The inside wall is 1 in. Cellotex⁴ and is glued to the outer wall of $\frac{1}{2}$ in. plywood. The two front doors overlap with thin Formica panels. A double-pane window is placed in one door for viewing. For temperature control we used a Bronwill heater⁵ bucking a Forma⁶ Cold Finger immersed in a polycarbonate tank. A floor-to-ceiling sonifier support is shown in Fig. 1. It is made of two aluminum discs (D_1) and (D_2) and a stainless-steel pole (D_3). The pole has a flat insert at the threaded end. By turning the pole in one direction, the unit can be taken apart. Turning the pole in the opposite direction tightens the support. The enclosure was tested and found free of ultrasonic leaks.

⁴ Armstrong Minatone-735, Washable, White.

⁵ Bronwill Scientific Division, Will Corp., Rochester, New York.

⁶ Forma Scientific Co., Marietta, Ohio.

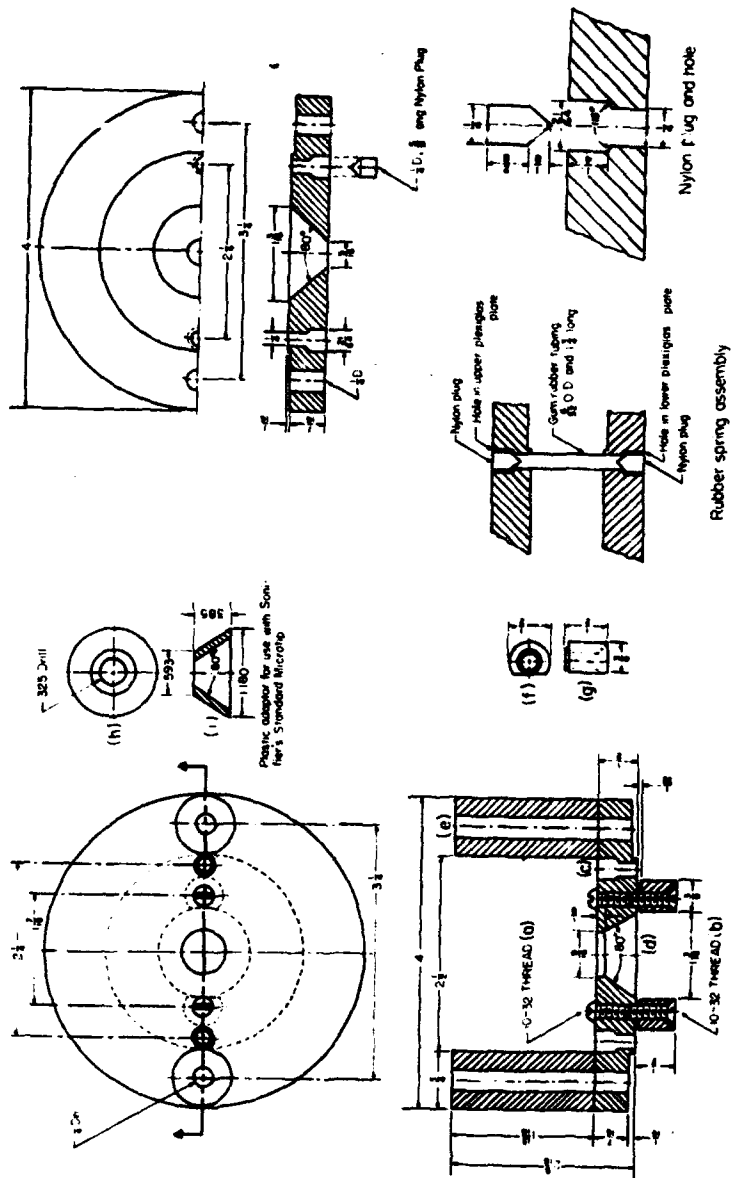


FIG. 2. *At left:* Diagram at top left is a cross-section and top view of the upper Plexiglas plate (A). Lower left diagram is a side view and cross-section of plate (A). Upper right diagram is a top view and cross-section of a plastic adaptor for a microtip. Lower right diagram is an end view and side view (cross-section) of the plastic aligners (J). (a) 10-32 thread, roundhead stainless-steel screw. (b) 10-32 thread, flat-screw (100°) stainless-steel screw. (c) Holes for rubber springs. (d) 80° angle opening for holding top of test tube containing solution. (e) Holes for stainless-steel rod I. (f) End view of plastic aligner (J). (g) Side view and cross-section of aligner (J). (h) Top view and cross-section of microtip adaptor. (i) Side view and cross-section of same. *At right:* Diagram at top is a top view, cross-section, of (one-half) the lower Plexiglas plate (B) and a side view, cross-section, of the same plate. The lower left diagram shows the details of the rubber spring assembly and the lower right view shows the cut of the nylon plug and the corresponding hole. *Fig. 2 continued on p. 230.*

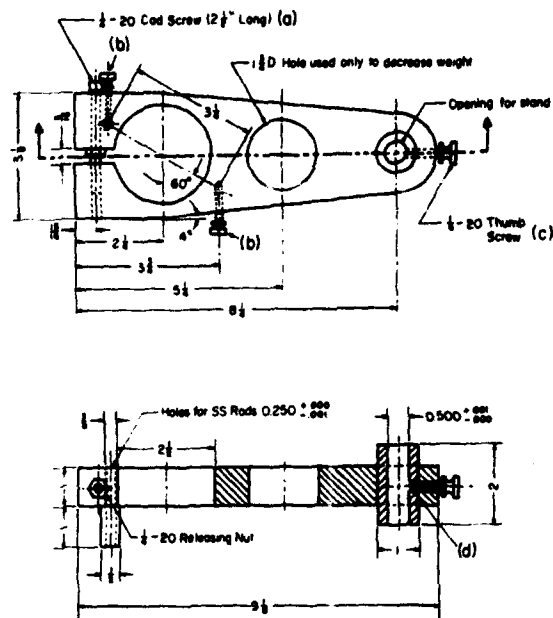


FIG. 2. *continued*. Upper diagram shows a top view and cross-section of the sonifier holder. The lower diagram shows a side view and cross-section of the holder.

Probe Characteristics

Sonifier probe ends become "greyed" with use (7,8) with only a slight decrease in emission intensity. However, a freshly polished probe was not used for the experiments reported here. The probe tip was "greyed" by sonicating in distilled water for three to four 15 min intervals at an output control setting of 3.2 (35 W) letting the probe cool for 10 min between sonications.

Materials

Beef heart lactic dehydrogenase (BHLDH) was obtained from Worthington Biochemical. Acrylamide gel electrophoresis showed two bands, the H_4 and H_4M isozymes. A specific extinction coefficient for LDH of 1.45 was used (9). Other chemicals used were reagent grade.

LDH was prepared by dialyzing 10 ml samples (0.3 mg BHLDH/ml) versus 200 ml 0.02 M phosphate buffer, 0.015 M NaCl, pH 7.0, overnight at 4°.

Activity determinations of LDH were performed at 37°. For sonication the standard stephorn was placed $\frac{5}{8}$ in. beneath the meniscus of the 10

ml samples. The glass vessels were 2" high $\times$ 1" wide and made of 2 mm Pyrex glass.

RESULTS

Under identical experimental conditions a polished probe destroyed 92% of the activity whereas a "greyed" probe destroyed only 70%. Several experiments showed changing losses of activity as the tip "greyed." Accordingly, we used "greyed" probes for all data shown in this report. The good reproducibility obtainable is shown in the following experiment. Six samples of LDH (0.01 mg LDH/ml) were sonified for 5 min each at output control setting 3.0 at 20°C. The average activity remaining was $27.4\% \pm 1.8\%$ (S.D.).

Effect of Probe Debris

The standard stephorn used in these experiments loses some of its metallic elements (7,8) in operation. The stephorn composition is 89% titanium, 7% aluminum, and 4% molybdenum. The probe debris did not appear to affect the activity of LDH since a solution containing horn debris did not inhibit unsonicated LDH. This debris might be significant for other materials, however.

SUMMARY

A self-aligning apparatus for use with a sonifier is described that permits reproducible ultrasonic treatment of solutions. The apparatus also reduces exposure of personnel to high-intensity sound.

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