ULTRASONIC FRACTIONATION OF CELLS IN MICROPLATES

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The effects of intense high-frequency sound waves traveling through a liquid were recognized by chemists Wood and Loomis in 1927. They published their observations regarding the effects of ultrasonics on emulsification, dispersion of colloids, fragmentation of small and fragile bodies, and fractionation of red blood corpuscles without using lysing enzymes or detergents. Although their work generated great interest, it was not until the mid -1950s, with the mass production of high-efficiency piezoelectric transducers, that high-power, low-cost ultrasonic equipment became readily available for research and industrial applications.

The two main products to use piezoelectric technology for high-intensity ultrasonic applications are ultrasonic baths and ultrasonic liquid processors. Baths work very well for their intended application-cleaning; however, their low, fixed and uneven intensity somewhat restricts their scope of utilization. Ultrasonic processors, on the other hand, are more versatile and are the instruments of choice for applications requiring predictable high-intensity ultrasonic energy.

Ultrasonic processors consist of three major components: an ultrasonic power supply (generator), a converter and a probe.

The ultrasonic power supply converts 50/60-Hz voltage to high-frequency (20-kHz) electrical energy. This voltage is applied to the piezoelectric crystals within the converter, where it is changed to small mechanical vibrations. The converter's longitudinal vibrations are amplified by the probe and transmitted to the liquid as ultrasonic waves consisting of alternate compressions and rarefactions. These pressure fluctuations cause the liquid to fracture in the rarefaction stage due to negative pressures, creating millions of microscopic bubbles (cavities). As the wave front passes and the bubbles are subjected to positive pressures in the compression stage, they

oscillate and eventually grow to an unstable size. Finally, the bubbles implode; creating millions of shock waves and eddies to radiate outwardly from the site of collapse, as well as generating extremes in pressures and temperatures at the implosion sites. Although this phenomenon, known as cavitation, lasts but a few microseconds, and the amount of energy released by each individual bubble is minimal, the cumulative amount of energy generated is extremely high.

Due to a variety of specialized accessories developed over the years, ultrasonic processors are now widely used in laboratories. Typical applications include: cell and tissue disintegration to access enzymes and metabolites; toxicity studies; sample preparation; enzyme linked immunosorbent assays (ELISAs); enzymology; protein purification for RNA, DNA and PCR labeling: hybridizations; receptor binding studies; preparations of liposomes; microemulsion; extraction; disaggregation; dissolution; catalysis of chemical reactions; and nanoparticle production.

Processing these applications with ultrasonics represents a quantum leap in productivity over earlier procedures. However, the methodology, which relies on manually inserting a single handheld ultrasonic probe into a test tube or well, becomes a laborious and time-consuming undertaking when working with microplates. To address this problem, a method was devised a few years ago that processes the samples indirectly by transmitting the energy through the microplate walls using a common ultrasonic bath or a specially designed tray horn that acts as an ultrasonic bath. While the intent was to process all wells simultaneously and identically, researchers have been less than enthusiastic with its reproducibility. This is due to the fact that most of the energy is absorbed by the microplate walls and microplate composition and tolerances vary greatly from manufacturer to manufacturer.

In the quest to increase productivity and improve repeatability, a 4-element probe was introduced in 1982 by **Sonics & Materials, Inc**. (Newtown, CT). More recently, to meet the high-throughput

requirement of genomics and proteomics laboratories, the company developed two additional multi-element probes.

For reproducibility, it is crucial that the intensity within each cell be uniform and predictable. Four element probes have been manufactured successfully by the company for 20 years; however, when they attempted to develop probes with eight and twenty-four elements, the prototypes performed below expectations in reproducibility. With Ultrasonics, the larger the area to be vibrated, the less likely it is for the amplitude to be evenly distributed. After producing a number of finite element analysis (FEA) models and spending many hours in the field, the company can now offer researchers viable time-saving tools that will impact favorably on their workload. Prior to assembly, each probe is checked to ensure that its resonant frequency and length are within tolerance. Once assembled, the multi-element probe is energized and checked again with fiber-optic instrumentation to ascertain that the excursion at the tip of each probe is as required.

The multi-element probes consist of a 20-kHz coupler and microtips that are designed to process 4, 8 or 24 deep well sample cells at one time; 1.5-2.0 ml microtubes; and 10-ml test tubes. A typical automated system incorporates a 750 watt microprocessor-controlled ultrasonic processor, a multi-element probe and an x-y positioning system. A processing cycle includes positioning the plate, energizing the ultrasonics for a predetermined duration, rinsing the probes, and repeating the procedure until all the sample wells have been processed. The system occupies minimal floor space, and once started has the ability to operate unattended until all the

plates have been processed. Because the duration of processing, level of intensity, and depth of immersion are highly controllable, the energy transfer into each well is inherently identical and repeatable, ensuring protocol duplication.

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