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Variation in performance at different positions of an ultrasonic VialTweeter – A study based on various physical and chemical activities

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ABSTRACT

Ultrasonic VialTweeter is used for the sonication of small volume samples. It contains a titanium block with 8 holes for vial insertion, to be used simultaneously for batch operation. In this investigation, the ultrasonic and sonochemical performance of ultrasonic VialTweeter has been evaluated at its different positions. Experimental results using calorimetry, ultrasonic capillary effect, sonochemiluminescence and degradation of Rhodamine B showed that the sonochemical activity differs greatly at different positions along the VialTweeter, with positions 3 and 4 showing the maximum efficiency whereas the positions 1 and 2 being the least effective positions. These results were further verified by acoustic pressure simulation, confirming that certain locations in the VialTweeter may not perform in the same way as others due to the variation in acoustic pressure at different locations.

1. Introduction

Ultrasonication is widely used for mixing, homogenizing, emulsifying and many other applications [1]. Ultrasound is commonly employed using an ultrasonic bath or ultrasonic probe, where the former passes ultrasound waves via a liquid medium, while the latter dissipates ultrasonic energy directly to the samples, which are usually in liquid/aqueous state [2]. An ultrasonic VialTweeter is similar to an ultrasonic probe. Whilst an ultrasonic probe works by immersing it directly to the liquid medium, a VialTweeter on the other hand, is attached to a titanium block with eight standard vial-sized holes, to create direct contact sonication with the vials. Small volumes of samples can be subjected to ultrasound with a VialTweeter by inserting standard Eppendorf vials into the holes in the titanium block for simultaneous batch sonication.

One of the advantages of an ultrasonic VialTweeter is to be able to sonicate samples in a vial without being immersed directly in a liquid medium. It contacts directly to the samples via the oscillating titanium blocks, hence producing higher intensity ultrasonic energy. Also, it surpasses the conventional ultrasonic probe as it lowers the risk of contaminating the samples in the liquid medium, since the samples are not in direct contact with the ultrasonic probe.

Ultrasonic VialTweeter has vast applications in the biomedical and pharmaceutical areas, where small volumes of samples are utilized. Lukic et al. [3] used an ultrasonic VialTweeter to extract digested glycans; Moest et al. [4] used it to rupture adipocytes cells; while others used it for dispersing bacteria [5], homogenizing fibrils [6], extraction of algae [7] *etc.* All the above reported studies using the VialTweeter have not looked into the efficiency of sonication at various positions of VialTweeter. On the other hand, Larguinho et al. [8] performed a detailed study where they compared the effects of using different ultrasonic systems (ultrasonic bath, ultrasonic probe, VialTweeter and a sonoreactor) on DNA fragmentation. They found that VialTweeter showed significantly lower performance in terms of energy output and DNA fragmentation [8] as compared to using ultrasonic probe and ultrasonic bath.

Considering the vast potential of using an ultrasonic VialTweeter in diverse applications and the lack of information regarding its performance, this investigation aims to evaluate the performance of an ultrasonic VialTweeter at various vial positions along the equipment. A series of characterization methods to evaluate the performance of the VialTweeter have also been looked into which include calorimetry [9], ultrasonic capillary effect [10], sonochemiluminescence [11,12] and degradation of Rhodamine B dye [12,13]. Besides, acoustic pressure simulation has been attempted to further interpret and validate the experimental results obtained.

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2. Methodology

All experiments were conducted using standard 1.5 mL Eppendorf vials (LGC Scientific Sdn. Bhd., Malaysia) and by employing an ultrasonic VialTweeter (24 kHz, 5-10 W, Hielscher Ultrasonic Technology, UIS250v, Germany). All chemicals were purchased from Sigma-Aldrich Malaysia and used without further purification. Fig. 1(a) shows an ultrasonic VialTweeter drawn using COMSOL Multiphysics 4.3. Statistical significance was assessed using Analysis of Variance (ANOVA) at 95% confidence level.

2.1. Calorimetry

The intensity of ultrasound delivered to individual vials kept at different positions of VialTweeter was measured calorimetrically [9], by calculating the heat dissipated into a known amount of water and dividing by the volume of the solution taken in the vial [13], whereby the power density at each position was obtained. Experiments were also performed at four amplitudes i.e. 50%, 55%, 60% and 65% over a period of 4 min. Fig. 1(b) shows different positions of VialTweeter along the titanium block. The power output of VialTweeter was examined by measuring the temperature rise of water in the vials. The rise in temperature was used to calculate the amount of energy dissipated which was then divided by the volume of solution taken in the vial to obtain the power density, as described by Manickam and Pandit [13].

2.2. Ultrasonic capillary effect

Capillary effect induced by ultrasonic waves was observed by inserting glass capillary tubes (OD: 1.0 mm, ID: 0.8 mm, WPI-Europe) at all the positions of VialTweeter. The capillary effect at each vial was measured by recording the difference in liquid height upon sonication for 30 s. With regards to the effect of thermal expansion on the capillary effects at each vial, though the experiment was not performed under an isothermal condition, care has been taken to ensure that the starting temperature of each experiment remained the same and that the experiment was only conducted for a maximum of 30 s for examination.

2.3. Luminol photography

Sonochemiluminescence images were recorded for 30 s with the vials in a solution of luminol [14]. Luminol solution was prepared by dissolving 1 mmol of luminol (3-aminophthalhydrazide, 97%), 0.1 mol hydrogen peroxide and 0.1 mol EDTA (ethylenediaminetetraacetic acid) in 1 dm^3 of 0.1 M sodium carbonate. The solution was adjusted to pH 12 by adding sodium hydroxide. Images were recorded on a digital single-lens reflex camera (Nikon D5100) body, using a macro lens of 60 mm focal length at an aperture of f, 2.8. The focal distance was adjusted to obtain an image ratio of 1:3, with an image resolution of 3872 × 2592 pixels (10.0 megapixels). The total intensity of emission was calculated after subtracting the background levels using ImageJ software [15], which was also used for further image manipulation over a fixed area of the vial.

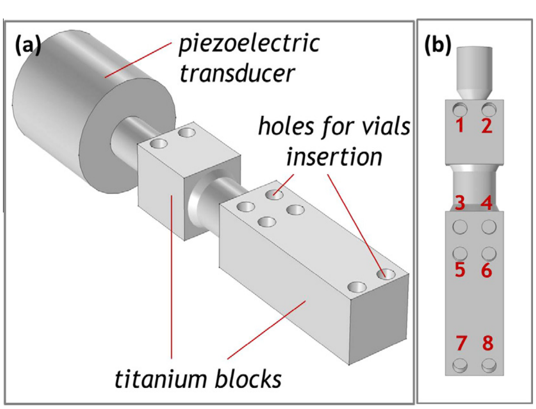

Fig. 1. (a) Ultrasonic VialTweeter with the titanium block of 8 holes for vial insertion; (b) positions of VialTweeter along the titanium block.

2.4. Degradation of Rhodamine B

5 mg L^{-1} of Rhodamine B dye solution was prepared and was inserted into Eppendorf tubes, which was then further inserted into different positions of VialTweeter, and was sonicated simultaneously for 30 min. The absorbance of the solution was then recorded every 5 min using a UV–Visible spectrometer (Varioskan Flash Multimode Reader, Thermo Fisher Scientific, USA) at 554 nm.

2.5. COMSOL simulation

Simulations were performed using the pressure acoustics frequency domain in COMSOL Multiphysics 4.3 and by considering titanium block as the medium in the model with the vials filled assumed to be filled with water.

2.5.1. Calculation of pressure amplitude

The power dissipated into VialTweeter was assumed to be the same as the input power, P_{US} of 250 W. The area of energy dissipation, A, was calculated based on the circumference of the individual vials by using the following Eq. (1),

$$A = 2\pi RL \tag{1}$$

where, R and L are the radius and length of the holes in the VialTweeter.

Intensity, I of the system can then be obtained via Eq. (2),

$$I = \frac{P_{US}}{A} \tag{2}$$

The acoustic pressure amplitude, $p_o(r)$, was calculated based on Eq. (3),

$$I(r) = \frac{p_0^2(r)}{2\rho c} \tag{3}$$

Upon rearranging, gives,

$$p_o(r) = \sqrt{\frac{2\rho c P_{US}}{A}} \tag{4}$$

where r is the spatial variable ($r = [x,y,z]$), ρ is the density of the medium and c is the sound velocity in the medium.

2.5.2. Acoustic pressure simulation

The acoustic pressure simulation was performed using a method as described previously [16] by applying the simplified Helmholtz equation as follows,

$$\nabla\left(-\frac{1}{\rho}\nabla p\right) - \frac{\omega^2}{\rho c^2}p = 0 \tag{5}$$

where, ω is the angular frequency.

The mesh generation used for this study was a predefined tetrahedral mesh with improved resolution at the curvatures, totaling up to 109,307 elements. The simulated results were validated by gradually increasing the mesh numbers until a negligible effect