Ultrasonic Trapping of Bioparticles Using Cylindrical Cavity

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ABSTRACT

In order to increase the ultrasonic trapping efficiency of bioparticles, a new cylindrical acoustic cavity with a concentric stainless cylindrical steel reflector for trapping and separation of bioparticles was designed and constructed. The resonant modes in this cavity were estimated by theoretical modeling and verified experimentally. For all bioparticles under study (RBC's, yeast and E-C0li), the simulation and measurement results were well agreed. Results obtained indicated that trapping efficiency reached 94% for RBC's at ultrasound intensity of 3 w/cm^2. Acoustic contrast factor changed according the density of bioparticles and suspended medium. So it is possible to separate particles with different densities.

Introduction

The sorting and trapping of target cells and suspended particles from a medium is of great importance to cell biology, drug delivery and related fields in biomedicine. Furthermore; the ability to separate and trap bioparticles is of profound interest to the biomedical community to obviate current clinical limitations.[1] The conventional regimes for cell separation, manipulation and trapping need not only expensive equipment but also the skillful management of equipment's. Sorting technology using acoustic waves has several benefits over the other methods such as mechanical,[2] biochemical,[3] magnetic,[4] and dielectrophoresis separation.[5] Ultrasonic manipulation technology has recently emerged as a powerful tool for handling micrometer-sized biomaterials (such as cells and bio-functionalized beads) in microfluidic chips.[6-10] It provides an accurate, low-power method of trapping that causes minimal damage to cells.[11] Also, acoustic standing wave manipulation is characterized by its long-range force field, determined by the pressure node spacing in the axial direction.[12] A number of studies indicated that the acoustic fields used caused no variations in particle integrity and cell viability in yeast, mammalian cells or erythrocytes. Various resonator systems are designed to exploit acoustic radiation forces originating from geometrically well-defined resonant ultrasonic fields to move particles or cells in suspension.[13-15] In the present work a simple acoustical driven cylindrical cell trapping technique was developed for manipulation and separation of biological cells. A theoretical analysis of the modulation of shock free resonances of standing waves in the space between cylindrical cavity and a concentric stainless cylindrical steel reflector was performed.

I.Theory

Ultrasound Radiation force theory

The fundamental theory on acoustic standing wave forces on particles has been described extensively. The force induced on particles in an acoustic standing wave field is the result of both the primary and secondary radiation forces, where the primary force originates from the standing wave and the secondary forces are due to sound waves scattered by the particles.[8, 11]

Primary radiation force (PRF).

Primary radiation force (PRF) can be divided into the axial component and the transverse component. The axial PRF acts in the direction of the propagation of the acoustic wave field and is stronger than the transverse one. The axial PRF translates particles or cells to either the nodes or the antinodes of the standing wave. The transverse PRF is subsequently responsible for packing the particles closer together and to withhold their positions[8]. The axial PRF, states that the acoustic force is proportional to the acoustic pressure amplitude P_0 squared and to the volume of the particle V_c. As the size dependency scales with the particle volume, the cube of the radius, the induced primary force is strongly dependent on particle size and thus, as the particle diameter is reduced, the acoustic force diminishes rapidly. The wave number is denoted by $4\pi/\lambda$ and x is the distance from a pressure node (equation 1).The acoustic contrast factor (φ) (equation 2), depends on both the particle density (ρ_p) and its compressibility (β_p) in relation to the corresponding properties of the surrounding medium (ρ_m, β_m).

$$F_{PRF} = -\frac{\pi P_0^2 \beta_m V_c}{2\lambda}\ \varphi(\beta,\rho).\sin\left(\frac{4\pi x}{\lambda}\right)$$

(1)

$$\varphi(\beta,\rho) = \frac{(5\rho_p - 2\rho_m)}{(2\rho_p + \rho_m)} - \frac{\beta_p}{\beta_m}$$

(2)

A most notable aspect of the acoustic contrast factor (φ) is the possible sign change depending on the densities and compressibility of the particle and suspension media, which determines the direction of the acoustic force and thus whether the particle will move towards the standing wave pressure node or the anti-node. Particles with positive contrast factor are driven toward pressure nodes, while they are driven toward antinodes for which contrast factor is negative.

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Because of the acoustic contrast factor, even particles which are neutrally buoyant, they can experience an acoustic force as long as the compressibility differs from the surrounding medium equation 2.[16,17] On the other hand; the compressibility (β) depends on both density and sound velocity, defined as

$$\beta = \frac{1}{\rho C^2} \tag{3}$$

The magnitude of the radiation force is proportional to the acoustic frequency and for particle manipulation it is advantageous to increase the difference in the mechanical properties between the particles and the host medium (density and compressibility) .[18] The cells are displaced from their random motion in the presence of a sound wave, and are set into oscillation by the frequency associated with the waves. The velocity of suspended cell depends on the host media density at constant US wave pressure (P). Thus, as the media density decrease the cell velocity increase equation (4). Indeed, the trapping bands of cells appear relatively faster at lower density media

$$P = \rho_m . C . \upsilon \tag{4}$$

Where, ρ_m is the density of the medium, C is the speed of sound, and υ is the cell particle velocity which is the root mean square of the instantaneous cell particle velocities over a time interval at the given position. In principle, for particles with size less than the wavelength (λ), they can be driven by the primary radiation force (PRF) generated in a standing wave field, thereby concentrated at either nodal or anti-nodal planes.

Secondary acoustic forces

When multiple particles in a suspension are exposed to a standing wave field, secondary forces caused by waves scattered by other particles will induced. Equation (5) shows the inter-particle forces, when the acoustic wavelength is much greater than the particle radius.[19]

$$F_B(x) = 4\pi r^6 \left[\frac{(\rho_p - \rho_m)^2 (3\cos^2\theta - 1)}{6\rho_m d^4} vc^2(x) - \frac{\omega^2 \rho_m (\beta_p - \beta_m)^2}{9d^2} p^2(x) \right] \tag{5}$$

Where, (r) is the radius of the particle, (d) is the distance between the particles and θ is the angle between the center line of the particles and the direction of propagation of the incident acoustic wave. I is acoustic wave intensity (square of the pressure amplitude P(x) in the x direction), $\acute{\omega}$ is angular frequency. The sign of the force is to be interpreted such that a negative sign means an attractive inter-particle force and a positive sign means a repulsive force. The influence of the secondary forces is usually very weak, due to the distance term d in the denominator, which means that it is only effective when the distance between particles is very small. The secondary force becomes important in aggregation and sedimentation applications, where particles initially are gathered in nodes by the PRF, and as interparticle distances become smaller the secondary forces assist in a further aggregation until the clusters finally become heavy enough for the gravity to overcome the buoyancy and start the sedimentation process.[19]

Cylindrical Cavity Resonator:

We shall consider standing waves in a fluid between two infinitely long coaxial cylinders. [20] The sound is generated in the fluid by a harmonic oscillation of the radius of outer cylinder, given by

$$r_b = \bar{r}_b - A_0 \cos \omega t^* \tag{6}$$

Where r_b is the instantaneous radius at time t^*, $\bar{r}_b$ is the mean radius, A_0 is an amplitude, and ω is an angular frequency of the harmonic oscillation. The inner cylinder is fixed at the center of the outer cylinder and thus a standing acoustic wave is formed in the fluid between the two cylinders when the driving frequency matches a resonance frequency of the system. The problem can be described only by two independent variables, the time t* from

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$$r = \frac{\omega r*}{c_0}, t = \omega t*, u = \frac{u*}{c_0} = \frac{*}{c_0^2}, \tag{7}$$

where C_0 is the speed of sound in the initial undisturbed fluid, u* is the radial component of the fluid velocity, □*is the velocity potential defined by $u* = \frac{\partial *}{\partial r*}$

Now, the most important parameter related to the geometry of the problem is the radius ratio. The radius ratio α is defined as

$$\alpha = \frac{r_a}{r_b} \qquad (0 \leq \alpha < 1) \tag{8}$$

where r_a is the radius of inner cylinder. The parameter α can be regarded as a measure of geometrical effect. In fact, when α is sufficiently small compared with unity, the wave amplitude at the inner boundary increases due to the focusing of cylindrical wave. The case that the inner cylinder does not exist is included in the present formulation as a limiting case of $\alpha \rightarrow 1$. On the other hand, if α becomes close to unity, with keeping the spacing between inner and outer boundaries constant, the wave motion becomes close to that of the plane wave. However, the treatment of the case of $\alpha \rightarrow 1$ requires some care, because the nonlinear plane wave resonance always leads to the shock formation if the dissipation effect is negligible, and the present method of analysis assumes the shock free resonant oscillations.

The advantage of cylindrical transducer is the simplicity and a strong focusing effect is also achieved, with high acoustic intensity in the center of the resonator .[19] If only waves propagating in the radial direction are considered, the expressions for the mean-square fluctuations of the pressure and the particle velocity are given by:

$$< p^2(r) > = \frac{p_0^2}{2} J_o^2(Kr) \tag{9}$$

$$< v^2(r) > = \frac{p_0^2}{2\rho_0^2 c_0^2} J_1^2(Kr) \tag{10}$$

Expression for the cylindrical force potential is given:

$$U(z) = \frac{p_0^2}{4\rho_0^2 c_0^2} (f_1 J_o^2(Kr) - \frac{3}{2} f_2 J_1^2(Kr)) \tag{11}$$

Corresponding radial force becomes

$$F(z) = \frac{p_0^2}{4\rho_0^2 c_0^2} [(2f_1 + \frac{3}{2} f_2) J_o^2(Kr) - \frac{3}{2} f_2 J_2^2(Kr)] J_1(Kr) \tag{12}$$

J_m are respectively the mth order cylindrical Bessel function where m =1, 2, 3.. The two non-dimensional factors f1 and f2 are given by:-

$$f_1 = 1 - \frac{\rho_m c_m^2}{\rho_p c_p^2} \qquad (13)$$

$$f_2 = \frac{2(\rho_p - \rho_m)}{2\rho_p - \rho_m} \qquad (14)$$
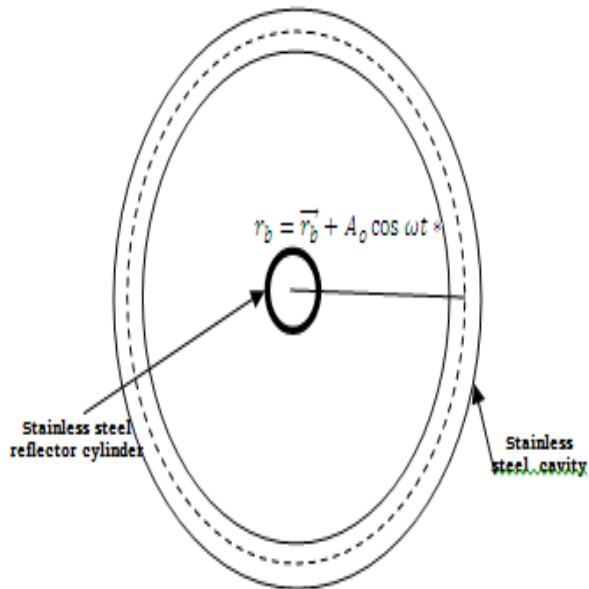

$$r_b = \vec{r_b} + A_0 \cos \omega t$$

Figure 1. Schematic diagram shows concentric cylinders. The acoustic standing wave is excited by an ultrasound wave oscillation from of the outer cylinder with amplitude A₀ and angular frequency ω.

In the two-dimensional case, the locations of particle trapping plane are the minimum force potential locations where the gradient of <U> is zero. Accordingly, particles will move to the positions where <p^2> =0. Once they are in the planes where <p^2>=0, there will be no contribution from <p^2> term. Then, the particles will move to the local points along the nodal lines and they form ordered patterns along the nodal line such as striations and aggregates.

II. Experimental Methods
Sample preparation
R.B.C's

Ten blood samples were obtained from healthy volunteers following a protocol approved by Medical Research Institute guidlines. Ten ml of blood was drawn and was anti-coagulated by traces of (EDTA). After plasma and Buffy coat were removed by centrifugation (800 rpm for 10 min.), the R.B.C's were washed in phosphate buffered solution (PBS) pH 7.4. The obtained R.B.C's were divided into small parts and diluted by different media e.g. phosphate buffered solution (PBS), agarose, and polyacrylamide gel to yield a final cell suspension containing 1.5x10^5 cell/ml. The cells concentration was counted using Neubauer haemocytometer. The relative viscosities for stock solution of 1 % agarose, and polyacrylamide gel media was determined relative to the phosphate buffered solution (PBS) using Zeitcfuchs cross arm viscometer by measuring the time spent by the solution to move a fixed estimated distance. The relative viscosity was calculated from the relation:- ($\eta_1 / \eta_2 = t_1 / t_2$) where η is the viscosity and t is the time.

Yeast samples

Dried yeast cells (*Saccharomyces cerevisiae)* were suspended in PBS pH 7.4 and diluted to yield a final suspension containing 1.5x10^5 cell/ ml.

E. Coli

E. coli obtained from the Department of Microbiology, Medical Research Institute, was suspended in PBS to prepare a final suspension containing 1 x 10^6 cells / ml. A mixture of R.B.Cs. and *E. Coli* cells 1:1 volume/volume was made and injected into the trapping chamber.

Ultrasonic trapping system

To investigate biological cells manipulation and separation, a simple homemade ultrasonic cell trapping system was designed and constructed. This system consists of three main parts: ultrasonic generator, cylindrical trapping cavity, and monitoring system.

Ultrasonic Generator

Ultrasonic Generator (Model CSL Shanghai, No822 Factory, China) operates at 0.8 MHz continuous wave mode with output intensity from 0.5 to 3 W/cm^2 was used as a source for ultrasonic waves. This instrument uses electronic tube to generate AC electric oscillation by means of a Calcium Zirconate Titanate circular transducer. The ultrasonic wave emitted from the transducer is coupled to the trapping cavity using an aluminum sheet extended inside the cavity.

Cylindrical trapping cavity

Trapping cavity (Figure 2) consists of a thin stainless steel evacuated cylinder of 0.5 mm height, 50 mm inner diameter and 60 mm outer diameter. The top and the bottom of the cylinder were covered with two circular glass slides. A cylindrical disc reflector of 0.5 mm thickness and 10 mm diameter was placed in the coaxial center of the evacuated cylinder. The two glass slides were sealed with epoxy resin. The outer stainless steel frame was placed in close contact with the US transducer. The ultrasonic waves were emitted from the transducer and travel through the cylindrical wall toward the center of the cavity and reflected back by the central stainless steel reflector to form standing waves. The cylindrical cavity described in the present work is different from that previously described by Wiklund and Hertz (2006a) [9] since a stainless steel reflector is placed at the center of the cylinder. This reflector make easy to align and can be operated as focusing mode that gathering the advantages of hemispherical resonator to this cylindrical one.

Light microscopic inspection monitoring

To study the behaviour of the biological cells inside the cylindrical trapping cavity under the effect of the ultrasonic waves, a light microscope system (Type: BX41; Olympus America Inc.) (Figure 3) was used. The trapping cavity was fixed over a microscopic stage, and nearly 2.5 ml of the cells suspension was injected inside the trapping cavity, then left for a minute to be get stable and was subjected to ultrasonic waves. The imaging capture system was consisted of CCD camera mounted on the light microscope column. The microscopic image was projected onto the CCD camera. The camera was connected to a personal computer (PC) across an interface Fly TV Prime3XTV tuner and video capture card 3.22. Images were recorded on the PC in order to perform imaging processing procedures. Recording of cell behaviour film under US effect was performed using Life View TVR program (version 3.21.400). At the beginning the of US application, the life view program was opened in the same time and by mouse curser on the PC screen, the film recording was started. Also images were captured by the snapshoot button.
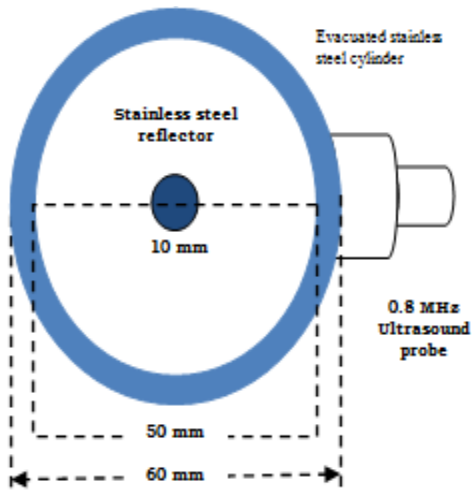
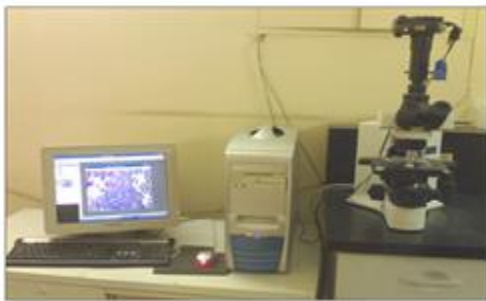**Figure 2. Schematic diagram of a home-made cylindrical trapping cavity.**

Figure 3. Photography of experimental set-up used for the light microscopy monitoring system.

III. Results and Discussion

Acoustic pressure simulation pattern

The pressure generated inside the cylindrical cavity containing RBC's suspended in saline is computed with the fast near–field method[21]. The numerical simulation was performed on a 64-bit computer with a 2.4 GHz dual-core processor and 4 GB of RAM. The optimization routine was implemented in MATLAB. The pressure simulation routines are written in C and called as C-MEX functions by MATLAB. Cylindrical cavity model and simulated pressure pattern obtained are shown in Figures 4 and 5, consequently.
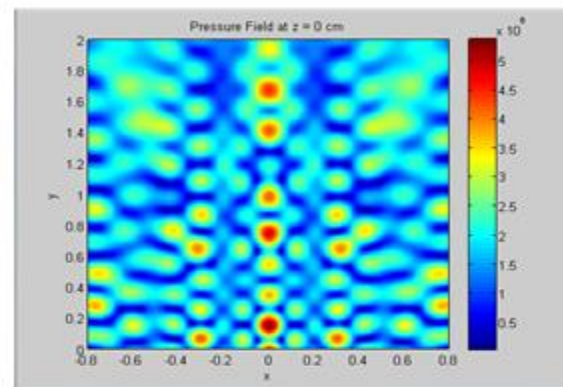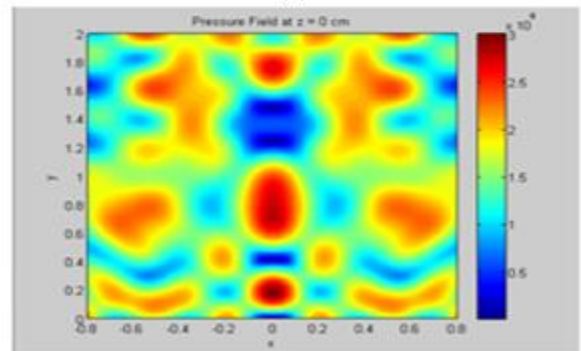

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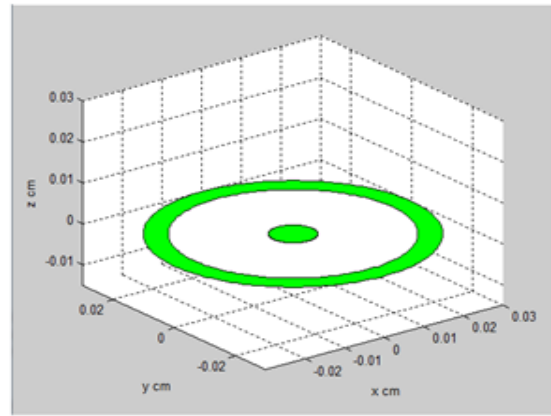
(b)

Figure 4. Cylindrical cavity model used in simulation in (a) x-y plan and (b) x,y and z.
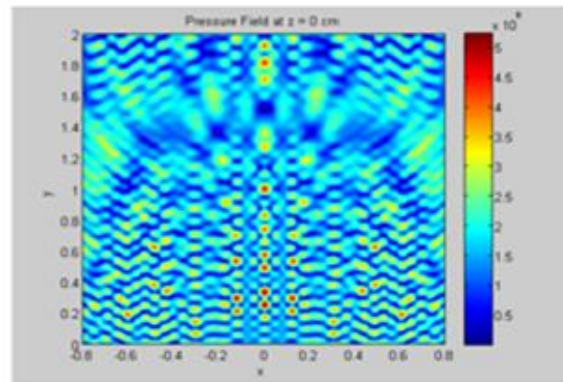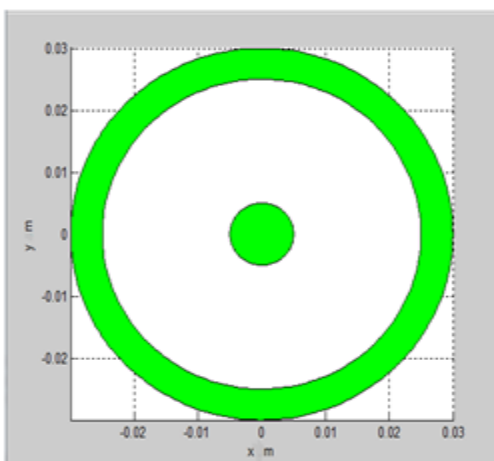

(a)

(b)

(c)

Figure 5. Pressure in the model under test contains RBC's suspension at a frequency of 0.8MHz simulation using grid finite difference of (a) =10^{-4} m, (b) =10^{-3} m and c= 10^{-2} m .

Acoustic pressure set up in a cavity cross section (x–y plane) by excitation at 800 kHz (Figure 5). In the color coding, red indicates the maximum value, blue the minimum. Particles collect at the location of minimum force potential, thus in a plane running along the centerline at 800 kHz and three planes (one along the centerline, two distant quarter of wavelength from the centerline) at 800 kHz. In this one dimensional standing pressure field these locations correspond to the pressure nodes. The acoustic radiation force pushes the suspended particles to the acoustic pressure node. The particles collecting at pressure nodes in the cylindrical trapping cavity, illustrated by the corresponding computed pressure distribution. The computed pressure nodes align with the experimentally observed collection lines (Figure 6).

Trapping pattern:

Light microscopy investigations (Figure 6) show the trapped pattern of the R.B.Cs. and Yeast observed after soncocation of nearly thirty seconds with 3 W/cm^2 ultrasonic waves in the trapping cylindrical cavity, the cells started to move toward the node positions due to the US standing waves interference forming trapping pattern. The brown color lines (Figure 6 a) are group of RBC's, the blue color lines (Figure 6 b) of stained yeast cells and the occurrence of white areas mean that the solusion was purified from the bioparticles. As shown in Figure 6, trapping pattern consists of a group of circular shapes. This pattern may be explained by the occurrence of wave interference of ultrasound emitted from the inner surface of the outer cylinder and the ultrasound waves from the inner surface of stainless steel cylinder. So the cells were concentrated in a random nodes position of the created standing waves. The result of the measurement agreed well with that of simulation. Both of the particles made very similar circular pattern.

When the ultrasonic waves of intensity in (W/cm^2) is applied to the cell suspension the cells suffer acoustic force F_{PRF} (N) which can be calculated for cylindrical trapping cavity using equation 11. When the standing waves are created within the cavity, variation in pressure points will occur including maxima and minima pressure points (nodes and antinodes) corresponding to velocity antinodes and nodes respectively.[22] The calculated acoustic contrast factor (Table 1) for RBC's, yeast and E-coli is defining whether a particle will move toward the pressure node or the antinode in the acoustic standing wave field. R.B.C's and yeast have positive contrast factors (φ) for medium concentration of 1.05 g/ml and E-Coli has negative contrast medium while for higher medium densities all types of cells (RBC's, yeast and E- Coli) have negative contrast Factors. The contrast factor is defining whether a particle will move toward the pressure node or the antinode in the acoustic standing wave field. A positive contrast factor results in movement toward the pressure node while a negative contrast factor results in a movement to the antinode [23]. The pressure is maximum at the antinode and minimum at the node. Due to this pressure differential, solid particles tend to reassemble and concentrate in zones of minimum acoustic potential energy, which is typically at the nodes [24]. If multiple nodes are present, they would be half a wavelength (λ/2) apart from each other. The acoustic force is depending on the physical properties of particles/ cells. The most important parameters, which affect the magnitude of this axial radiation force, the major force component in acoustic standing wave manipulation, are density ρ , speed of sound c, and the radius R
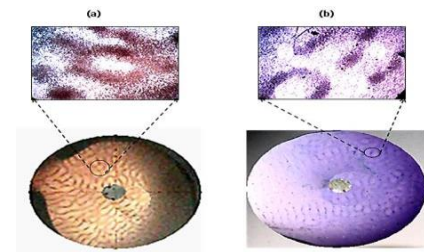

Figure 6 . Light microscopy image of (a) RBCs and (b) stained yeast after sonocation with 3 W/cm^2 US for 30 s in concentric cavity.

Table 1. Acoustic contrast factor of bioparticles (RBC's, Yeast and E-Coli) at different media concentration.

Media density (g/ml)	Acoustic contrast factor (φ)		
	RBC's	Yeast	E-Coli
1.05	0.090	0.056	-0.099
1.1	-0.002	-0.037	-0.197
1.125	-0.047	-0.083	-0.245
1.15	-0.092	-0.128	-0.293
1.25	-0.269	-0.307	-0.481
1.3	-0.355	-0.394	-0.573
1.35	-0.441	-0.481	-0.663

Effect of ultrasound intensity on trapping efficiency

In the present study, the effect of different ultrasound intensities, suspending media concentration and type of bioparticles on the trapping efficiency, trapping time and trapping velocity of the bioparticles were done.

Cell trapping fraction

Cell trapping fraction is defined as the percentage of the trapped cells after constant time. Captured images (at 400 x) were processed by using Scion image program version 4.0.3.2 to calculate cell trapping fraction. Cell trapping fraction was calculated from the relation:

$$Cell\ trapping\ fraction = \frac{mean\ no.of\ trapped\ cells/image}{mean\ no.of\ cell/image} x\ 100\%$$

(15)

Figure (7) shows the effect of ultrasound intensity on both the trapping efficiency of RBC's and the time needed to trap all cells in field. For a trapping time of 30 s, increasing of US intensity increases the percentage of cell trapping fraction per image studied. That is an indication of increasing in trapping efficiency. Trapping efficiency up to 94% was obtained at a sonication time of 25 s at an ultrasound intensity of 3 w/cm^2
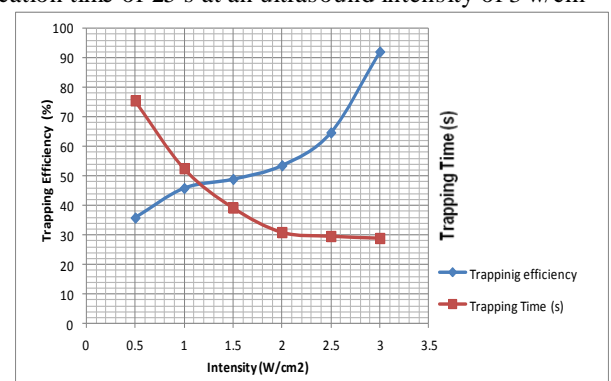

Figure (7) . Affect of ultrasound intensity on trapping fraction of RBC's and trapping time.

**Effect of suspending media concentration on trapping behaviour**

To study the effect of suspended medium concentration on trapping behaviour (cell trapping velocity and time) of R.B.C's. different concentration of RBC's suspensions were prepared at phosphate buffer solution (PBS). Agarose and polyacrylamide gel were added to form a gel media of concentration ranged from 0.063 to 1 %. As shown in Figure (8) for all particles increasing of medium density tends to decrease in acoustic radiation force.
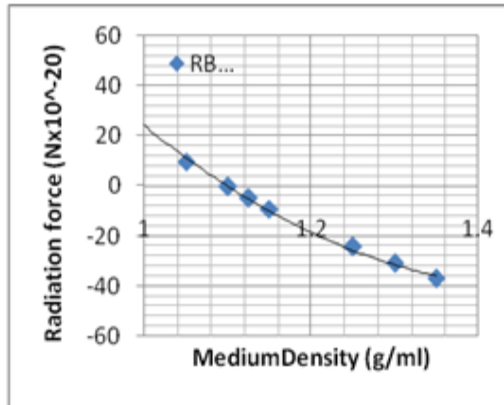
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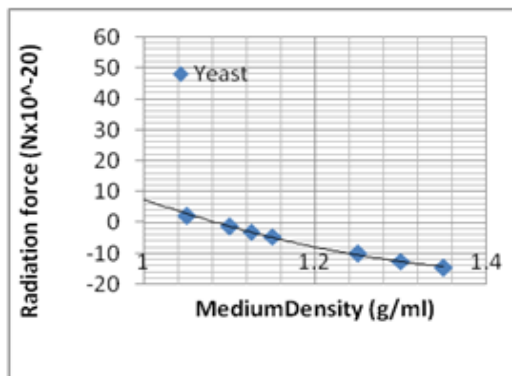
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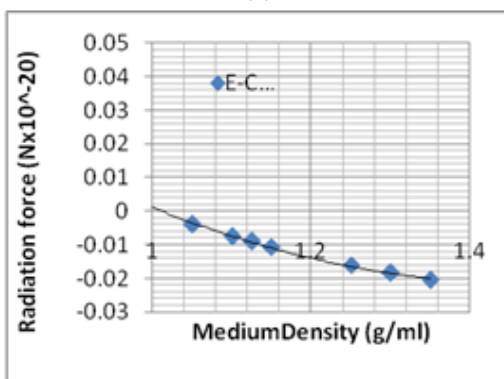
(c)

Figure 8. Effect of medium density on the bioparticles acoustic radiation force.

IV. Conclusions

In this study, standing wave fields, radiation force, and trapping patterns of bioparticles (RBC's, yeast and E-Coli) in cylindrical ultrasound cavity with concentric stainless steel cylinder ultrasound as reflector were simulated and the results were compared with those of measurement. The device was designed and the effects of varying experimental parameters were investigated. A two-dimensional FE model was used to predict the shape and amplitude of the pressure field in the device. For RBC's, yeast and E-Coli the simulation and measurement results were well agreed. Study indicates that increasing ultrasound intensity increases the trapping efficiency of bioparticles and decreases the time consumed to trap bioparticles. Increasing of trapping media concentration tends to increase the trapping time and decrease the cell velocity. The results of this study showed the possibility using by cylindrical standing waves in trapping of bioparticles.

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