



Measuring the Average size of Ultrasound irradiated Human Erythrocytes using Laser Diffraction Technique

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ABSTRACT:

Ultrasound technology is widely used in clinical applications. In the present paper, the subtle changes in the Size of Human Erythrocytes at the cellular level due to ultrasound irradiation were studied by employing Laser Diffraction Technique. The Laser Diffraction Technique is a very rapid and simple tool for assessing the size of Erythrocytes. The Human blood samples were collected from healthy donors each time an experiment was performed. Blood was then transferred to heparin anticoagulant tubes to prevent it from coagulation. The Collected blood samples were then irradiated with ultrasonic standing waves at a frequency of 3 MHz for a duration of 1 Hour at an interval of 30 minutes using an Ultrasonic Interferometer in an identical and controlled environment. The data reveals variation in the size of ultrasound irradiated Human Erythrocytes when compared with that of Control Human Erythrocytes.

Keywords: Size and shape, Erythrocytes, Ultrasonic Interferometer, Laser diffraction technique

1. Introduction:

Ultrasonic Biophysics is the study of the process that governs how ultrasound interacts with biological materials. The study of the bio-effects of ultrasound on biological materials can lead to therapeutic applications and risk evaluations for diagnostic ultrasound applications [1]. Therapeutic applications of ultrasonography in medicine have been recognized and were useful for many years. [2]. Ultrasound is frequently utilized as a therapeutic tool in lithotripsy of kidney stones, physiotherapy, tissue destruction in case of tumor treatment, and as a surgical tool in the form of high-intensity focused ultrasound. It's also been used as a drug delivery system [3]. After irradiating ultrasound to human blood the authors then opinioned that the acoustic impedance values of plasma, cell membrane, and hemoglobin must be considered when studying the absorption of ultrasound in blood and its component [4]. According to the latest research on ultrasound, Biomedical Ultrasound has a lot of potential for novel applications and medical improvements. A deeper understanding of the process of tissue contact with ultrasound is essential to progress in the therapeutic use of ultrasound. The first biological effect of ultrasound was reported by Langevin in 1917. Ultrasonic absorption was seen in biological fluids like water, and milk at 1 -10 GHz using the phenomenon of damped oscillation [5]. The influence of ultrasound on the living system was thermal and mechanical destruction. The erythrocytes in the isotonic suspension were destroyed when treated with ultrasonic radiation [6]. Changes in biophysical parameters such as refractive index, conductivity, and pH of Human blood irradiated to ultrasound have been observed [7]. Ultrasound irradiated to human blood in vitro resulted in changes in the rheological parameters such as Viscosity, surface tension, and Volume flow rate [8]. Changes in the size of erythrocytes were reported due to electromagnetic radiation produced by mobile phones [9]. By employing the laser diffraction technique, the size of erythrocytes of different diseases was also determined [10].

The concept of using laser diffraction to measure Erythrocytes dimensions was first introduced by Johnson [11] who noted that RBC membrane dimensions may be measured by using commercially available ektacytometers, a device that uses a laser in counting applications. This machine was designed by the Bessis group at the institution de pathologie cellulaire in 1975 [12]. Erythrocytes' radius was previously measured using a laser diffraction technique, and the findings were quite precise when compared to other biological measuring techniques used to determine the RBC deformity [13,14]. Based on this background, our work aimed to Utilize Laser Diffraction Technique and determine the effect of ultrasound irradiation on the average size of human erythrocytes.

Materials and Methods:

Blood Sample Collection and storage

The study was performed following the procedures formulated by the National Ethical Guidelines for Biomedical and Health Research by the Indian Council of Medical Research, New Delhi, and certifies that the studies on human blood in vitro were carried out in accordance with the principles of the declaration as laid down in the 1964 Declaration of Helsinki. Human blood samples of volume 5 ml were collected from healthy donors with their consent, each time an experiment was performed. The collected blood sample was then transferred to Heparin tubes to prevent it from coagulation. A total of 10 Blood samples were collected from different subjects to study the effect of ultrasound irradiation on the size of erythrocytes [7].

Exposure of Ultrasound

A variable path ultrasonic interferometer (Figure 1: Mittal Enterprises, Model M-81) of frequency 3 MHz has been utilized for irradiation. The blood sample to be irradiated to ultrasonic standing waves was filled into the measuring cell. The high-frequency generator excites the quartz crystal present at the bottom of the measuring cell at its resonant frequency which generates ultrasonic waves of 3 MHz frequency in the Human Blood sample. The sample was irradiated for a duration of 1 Hour at an interval of 30 minutes [7].



Figure 1: An ultrasonic Interferometer of 3 MHz frequency used for the purpose of irradiation.

Laser Diffraction Technique for determination of the size of the erythrocyte

In the present study, the laser diffraction technique is utilized for finding the average size of normal and ultrasonically irradiated Human Erythrocytes. Figure 2 gives the schematic experimental arrangement. Figure 3 shows the experimental arrangement of

Laser Diffraction Technique for determining the Average Size of Human Erythrocyte

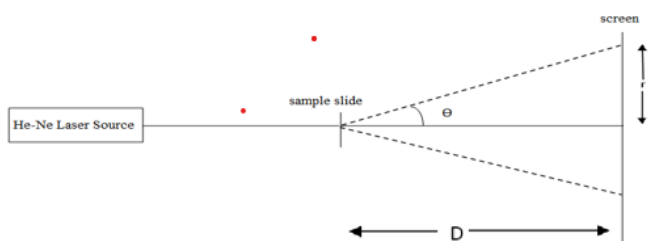


Figure 2 Schematic Diagram of Laser Diffraction Experiment

the function of the diffracting particle's size, shape, and orientation of the particles concerning the direction perpendicular to the incident beam. The radius 'r' has been calculated for the various values of 'D'. A plot between the radius of the diffraction and the sample distance from the screen was drawn, and the tangent of an angle of diffraction was obtained from the slope of the plot. [9, 10]. The average size of normal and ultrasonically irradiated human Erythrocytes was then calculated using the equation

$$d = \frac{1.22\lambda D}{r}$$

Here

$\lambda = 6328\text{\AA}$, the wavelength of laser light used.

D = distance between the screen and the sample.

r = first-order diffraction radius pattern.

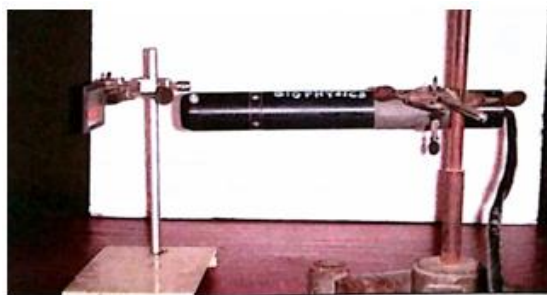


Figure 3: Experimental Setup

2. Result:

Table 1 reveals the data on the Average Size of Human erythrocytes of Normal and ultrasound irradiated samples for a duration of 1 h at an interval of 30 minutes for 10 samples. Here 0 Min represents the size of the erythrocytes before Ultrasound irradiation. The percentage variation in the value of the average size of ultrasound irradiated Erythrocyte for a duration of 1hr is 25.70 %

Table 1 Data on Average Size of Human Erythrocytes of Ultrasound Irradiated Samples

S.No.	Average size of Erythrocytes (μm)			% variation for 60 min
	0 Min	30 Min	60 Min	
1	7.14	8.11	11.3	58.26
2	7.72	9.32	10.4	34.71
3	8.21	9.78	9.65	17.53
4	8.04	8.12	8.39	04.35
5	7.70	9.00	10.29	33.63
6	7.72	8.31	9.00	16.58
7	8.30	9.20	9.69	16.74
8	6.01	7.89	9.08	51.08
9	7.95	8.05	8.39	05.53
10	7.04	7.37	8.35	18.60
Mean	7.58	8.51	9.45	25.70

Figure 4 shows the comparison of Average size of Human Erythrocytes with respect to the time of ultrasound irradiation.

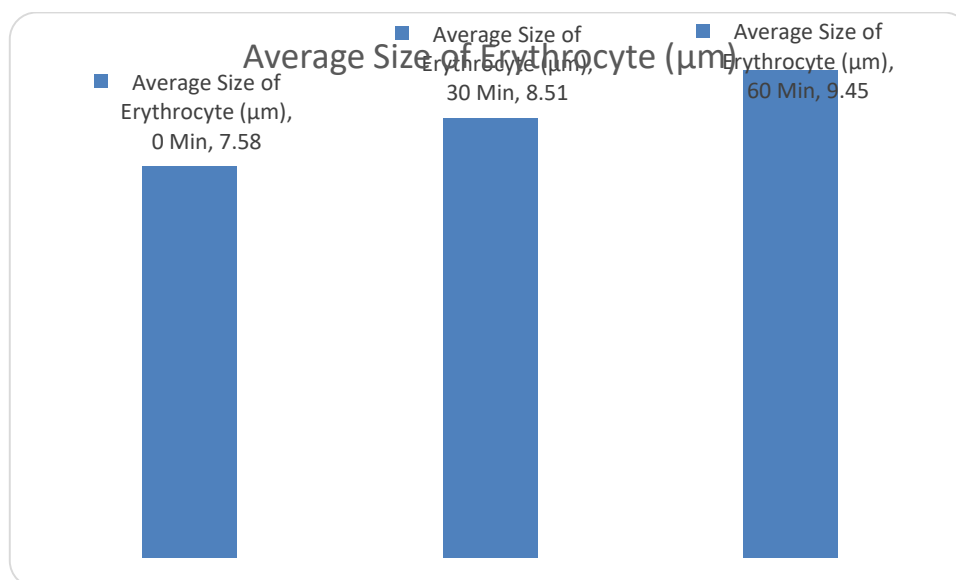


Figure 1 A Comparison on Average Size of Human Erythrocytes

3. Discussion:

Changes in cell division capacity, and membrane permeability are a few of the bioeffects that ultrasound may have on cells. The size of the erythrocyte plays a crucial role in the normal functioning of the human biological system. The precise information regarding the size of erythrocyte may be an important aspect for determining the deformability while flow through different micro-channels, osmotic resistance, and their oxygen-carrying ability. To determine the disease in humans, the information regarding the size of the erythrocyte may play a vital role. Even though different instruments like microscopes are available for determining the size of erythrocytes, a very simple technique known as laser diffraction technique developed at the lab has been adopted which gives the average size of an erythrocyte.

The present study mainly indicates the impact of ultrasound on the Erythrocyte membrane which in turn alters the dimension of the erythrocytes. It was observed that the average erythrocyte size of the Ultrasound irradiated group was significantly different compared to the control group. These alterations may be related to how ultrasound affects the properties of the erythrocyte membrane. The ultrasound irradiation has affected the human blood at the cellular level which might have caused an increase in the size of an erythrocyte. The increase in the size of erythrocytes may be due to the hypertonicity of the medium [9]. These changes in the size could relate to impairment of sodium and potassium pump mechanism which can lead to loss of the RBC membrane permeability, which caused RBC to lose or take up the materials and liquids resulting in decreasing or increasing their sizes [14]. Ultrasound irradiation might have affected the erythrocyte membrane elastic property due to which the average size of the erythrocytes is found to increase.

Conclusion:

The Laser Diffraction Technique can be used in determining the average size of erythrocytes. In this study, The average size of Human erythrocytes is found to be increased due to ultrasound and the change in size could relate to the impact of ultrasound on the erythrocyte membrane. The results obtained in the present work can be used in the future for studying the biological effects of ultrasound and interpreting the in vivo effects.

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