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ULTRASONIC INVESTIGATION ON HUMAN GALLBLADDER STONES

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ABSTRACT

The present investigation aims to determine the acoustical parameters of gallbladder stones using Pulse-echo-overlap technique to optimize a disintegrator design. The Ultrasonic velocity for different types of gallbladder stones was measured at 5MHz. From the measured values of Ultrasonic velocity (U), the Specific acoustical impedance (Z), Debye's temperature (θ_d), Elastic modulus (E) and Acoustical attenuation coefficient (α) were calculated. These measured parameters are helpful for the stone fragmentation by a disintegrator using Extracorporeal Shock Wave Lithotripsy (ESWL). The stone fragmentation depends on its mechanical properties such as hardness. It may be used to select an optimal frequency with appropriate intensity required for stone fragmentation. The tightness of molecular binding is determined by calculating the Elastic stiffness constant (C_{11}) which varies among the types of gallbladder stones.

KEYWORDS: Gallbladder Stone, Hardness, Stone Fragmentation and Ultrasonic Velocity

INTRODUCTION

Ultrasound is a non-invasive diagnostic and therapeutic tool used in modern medicine to examine internal organs without surgery. Ultra sonography is very safe as it does not involve hazardous radiation such as X-Rays. The recent technological advancements such as Endoscopic mechanical lithotripsy, Endoscopic electroscopic electro hydraulic lithotripsy, Endoscopic ultrasound and Laser lithotripsy methods have received attention in the field of medicine for gallbladder stone fragmentation [1-4]. Gallbladder stone remains a serious health concern for human beings, affecting millions of people throughout the world [5, 6], which could lead to dangerous consequences if left untreated. As on today, surgical removal of the gall bladder is the only solution available to treat the gallbladder stone disease [7]. The need of the hour is to develop some device or technique to increase the rate of fragmentation of the stones so that this condition can be treated without the need of surgery. Though many studies on gallbladder stone samples were carried out [7-9], so far there are not many investigations done on fragmentation of gallbladder stone using Ultrasonic technique. Hence, an attempt has been made to investigate ultrasonic technique as a tool to design a disintegrator to fragment the gallbladder stone.

The composition of gallbladder stones are complex and diverse, the mechanical properties greatly depend on its composition. The mechanical properties such as hardness of a material depends on impurities, dislocations, vacancies, temperature, composition, Lattice energy, Debye's temperature, and Inter-atomic spacing [10]. The hardness of the samples carries information about the molecular binding, elastic constants of the material, etc [11-12]. Vickers hardness test is used to find the hardness of different types of gallbladder stones.

It is reported that indentation method for testing hardness is widely used to describe numerous material parameters in the field of research and development [13]. The Vickers indentation is a common method used to characterize the hardness of materials. The Vickers hardness test is a non-destructive method for calculating the hardness of a bulk material of lightweight. Also, Vickers test is easier to use than other hardness tests, since the required calculations are independent of the size of the indenter and the indenter can be used for all materials irrespective of hardness [14].

The mechanical behavior of a stone may be helpful to fragment a particular type of stone group with appropriate energy. It prevents the damage of gallbladder tissues due to excess energy exerted by shockwaves. Further, the study would help in the development of better lithotripters with better efficiency. ESWL (Extra Corporeal Shock Wave Lithotripsy) provides an alternative to surgery and for better ESWL procedure, identification and strength of gallbladder stone type may be useful. The fact that the range of hardness found for gallbladder stones may help to explain the observed difficulty for the fragmentation of a specific gallbladder stone [15]. Hence, we made an attempt to develop a disintegrator with suitable shock wave frequency with an intensity to fragment the different types of gallbladder stone.

MATERIALS AND METHODS

The gallbladder stone samples were collected from the Department of Digestive system diseases in Selvarangam hospital, Anna Nagar, Chennai, Tamilnadu, India. The Ultrasonic velocity measurement for the different types of gallbladder stones was done using an Ultrasonic Time Interval ometer (Model UTI-101, Innovative Instruments, Hyderabad, India). It is based on Pulse-echo-overlap technique coupled with a dual trace Oscilloscope of 30 MHz (Model M3716, Aplab). The Ultrasonic velocity (U) measurement was calibrated with glass as a reference. The uncertainty in the measurement of Ultrasonic velocity was within ± 1%. To provide a good contact between gallbladder stone samples and the transducer (5MHZ 100PZT-VD Transducer), the gallbladder stones samples were cut using a razor blade to provide opposing flat parallel faces. The principle of measurement is to make the two signals of interest to overlap on the oscilloscope. Two prominent echoes are selected for overlapping as shown in Figure 1.

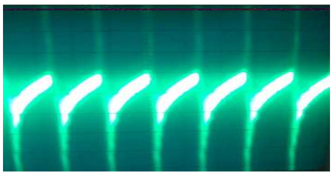

Figure 1: Echo Train in the CRT

From the measured values of Ultrasonic velocity, the following parameters were obtained as given below.

- **Specific Acoustical Impedance (Z):** It is defined as the resistance offered to the propagation of the ultrasonic wave in a material. For a given material, it depends on the physical properties of the material and is independent of the wave characteristics and frequency.

  Specific acoustical impedance (Z) = Uρ kgm^{-2}s^{-1} [16]

  Where, ρ is the density of the gallbladder stones sample (ρ = m/v) and U is the ultrasonic velocity.

- As the ultrasonic wave travels with certain velocity, the waves are absorbed. This leads to thermal effects in the biological system. These thermal effects are expressed using a characteristic temperature known as Debye's temperature.

Debye's temperature (θ_d) = $hk^{-1}(3N_pP/4\Pi V)^{1/3}U$ [16, 17]

Where, h is the Planck constant, k is the Boltzmann constant, N_P is the Avogadro's number, V is the volume calculated from the effective molecular weight and the density (M/ρ), P is the Number of atoms in the molecular formula and U is the ultrasonic velocity.

- **Elastic Modulus (E):** It is defined as the material or component's tendency to be deformed elastically when a force is applied on it.

  Elastic modulus (E) = $U^2 \rho$ kg $m^{-1}s^{-2}$ [16, 18]

- **Attenuation Coefficient (α):** It is related to the total loss of sound by any means, including scattering and is more relevant towards the prediction of overall transmission of ultrasound as it propagates through tissue. Attenuation is defined as the rate of decrease of energy when an ultrasonic wave propagates through a medium. The level of attenuation is measured by the change in intensity or amplification in terms of decibel dB. The amplitude of the wave decreases along the x-direction from the source as given by the relation: $A=A_0 e^{-\alpha x}$, Where A be the amplitude of the ultrasonic wave propagating through a medium, A_0 is the amplitude of the wave when the distance x is equal to zero and α is known as the attenuation coefficient. Attenuation coefficient is defined as attenuation per unit length.

  Attenuation coefficient (α) = $20d^{-1}(\log_{10} A_0/A)$ dB/unit length [16]

  Where, A_0 and A are amplitudes of the reference signal (in V) with and without the gallbladder stone respectively.

  A_0 and A are displayed simultaneously on the Oscilloscope; d is the total distance traveled by the ultrasonic wave registering between two echoes.

Hardness measurements were done using a Shimadzu HMV-2T Tester at Archbishop Casimir Instrumentation Centre (ACIC), St. Joseph College, Trichy, Tamilnadu. A 10 mm diameter surface gallbladder stone sample was dried to remove moisture content present in it. The Vickers hardness test method consists of indenting the test material with a diamond indenter which is in the form of a right pyramid with a square base and an angle of 136 degrees between the opposite faces. The indented test material is then subjected to a load of 1 to 100 Kg F. Vickers diamond pyramid indenter was applied on the sample for a period of fifteen seconds with loads of 25, 50, 100 and 200 grams. The indented surfaces of samples exhibit the formation of cracks at around P>200 grams. Such cracks are typical for a brittle material. H_v (Vickers hardness value) increases with load up to 200 grams and then reaches a plateau, emphasizing the fact that 200 grams is the ideal load to investigate gallbladder stone micro hardness [19]. The cross section of Vickers pyramid is a square and the depth of indention corresponds to one seventh the diagonal of the square shaped indention produced on the sample. The two diagonals of the indentation impressions after removal of the load were measured using the calibrated micrometer attached to the eyepiece of the Leitz metallux II microscope at 40x magnification and the average was calculated. The area of sloping surface of the indentation was calculated. The data obtained by measuring the diagonal points was converted into H_v units.

- Vickers hardness values (H_v) was calculated using the relation,

  H_V = 1. 8544 x (P/d^2) Kg mm^{-2} [20]

Where, P is the Applied Load in kg., d is the mean diagonal length (in mm) and H_v is the Vickers hardness number.

- Elastic stiffness constant gives an idea about the strength and tightness of bonding between the neighbouring atoms and is calculated using Wooster's empirical formula,

$C_{11} = (H_V)^{7/4}$ GPa [21]

RESULTS AND DISCUSSIONS

Ultrasonic Velocity Measurements

The Ultrasonic velocity measurement for different types of gallbladder stones was measured using an Ultrasonic time Interval ometer based on the Pulse -echo -overlap technique coupled with dual trace oscilloscope. The density of these stones was measured and calculated using the formula: density = mass/volume. Based on the mean ultrasonic velocity and density values, other acoustical parameters such as Acoustic impedance, Debye's temperature, Elastic modulus and Attenuation coefficient have been derived and summarized in Table 1.

Table 1: Mean Values of Ultrasonic and Acoustical Properties of Gallbladder Stones (5 MHz, Room Temperature)

Type	Gallbladder Stones Colour	Ultrasonic Velocity (U) (ms^{-1})	Density (ρ) (Kgm^{-3})	Acoustic Impedance (Z) $x10^5$ ($Kg\ m^{-2}\ s^{-1}$)	Debye's Temp (θd) K	Elastic Modulus (E)$x10^{10}$ ($kg\ m^{-1}s^{-2}$)	Attenuation Coefficient (α) dB cm
Cholesterol	White	0762	0584	4.4	084	0.034	0.7306
Cholesterol	Whitish Brown	0834	0899	7.5	109	0.062	0.7501
Bilirubinate	Brown	1159	0819	9.5	147	0.110	0.7985
Mixed	Dark Brown	1510	0739	11.1	185	0.168	0.7128
Mixed	Black	2413	0881	21.3	334	0.513	0.5371

The overall variation in the ultrasonic parameters measured and calculated for the various types of gallbladder stones is presented in Figure 2 as a three layer plot.

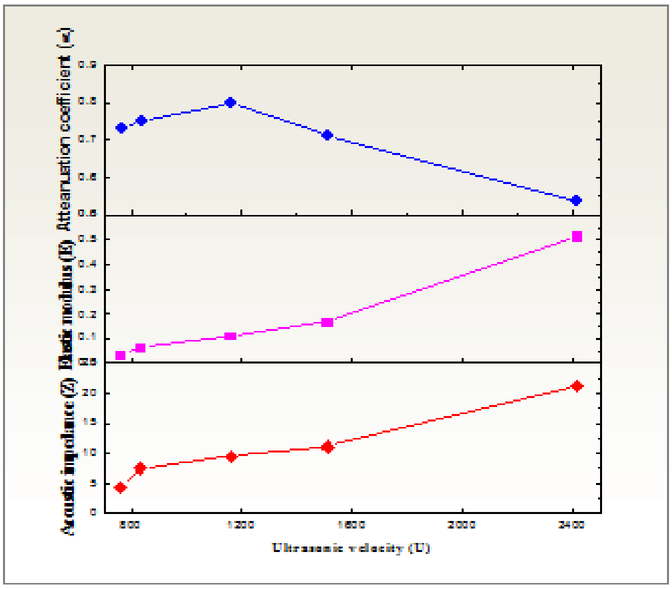

Figure 2: Trend Graph of Ultrasonic Velocity (U) versus Attenuation Coefficient (α), Acoustic Impedance (Z) and Elastic Modulus (E)

From the Table 1, it is observed that the ultrasonic velocity and other derived acoustical parameters of different gallbladder stones decreases in the following order: Mixed (Dark brown & Black) > Bilirubinate (Brown) > Cholesterol (White &Whitish brown) gallbladder stones. The variation in the calculated acoustical parameter values was due to the variation in the chemical composition of each gallbladder stone. Gallbladder stones with higher values of Ultrasonic velocity, Density, Acoustic impedance, Debye's temperature and Elastic modulus indicates its hardness. Hence, it is difficult to break. Therefore a high energy shock wave is required to break it into fragments. It is inferred from Table 1 that Cholesterol stones are soft in nature and it is confirmed by its ultrasonic velocity values (0762 & 0834 m/s). The Attenuation coefficient of mixed type black color gallbladder stone is less (0.5371 dB cm). This has proved that it is harder which is confirmed by a higher elastic modulus value (0.513 x 10^{10} kg $m^{-1}s^{-2}$)

The higher values of Elastic modulus do not produce an appreciable strain even though a higher stress is applied: The Mixed type black colored gallbladder stone is the hardest one due to its higher content of calcium carbonate.

The higher Debye's temperature value also suggests that Mixed type gallbladder stone is hard in nature. Also, the ultrasonic velocity of Mixed type black colored gallbladder stone is high (2413 ms^{-1}) which proves that its hardness and it is confirmed by a less acoustical attenuation coefficient (0.5371 dB cm). Thus, Mixed type gallbladder stones are found to be slightly more difficult to break in comparison to other types of gallbladder stones [22].

Hardness Measurements

Hardness is a measure of the resistance to plastic deformation [23]. This permanent deformation can be achieved by indentation, bending, scratching and cutting. Hardness of a material depends on its impurity, dislocations, vacancies, temperature, composition, etc. It gives information about the molecular binding, elastic constant of a material. Vickers indentation is a common method used to characterize the hardness of materials.

The mechanical property of a gallbladder stone has been studied using Shimadzu HV-2T Tester fitted with a Vicker diamond pyramidal indenter. The measured mean hardness values of the different gallbladder stones are given in Table 2.

Table 2: Mean Hardness Values for All the Three Types of Gallbladder Stones by Vickers Hardness Test Method

Applied Load (in Gram)	Cholesterol Stones (White & Whitish Brown Stones)	Bilirubinate Stones (Brown Stones)	Mixed Stones (Dark Brown & Black Stones)
25	6.63	8.16	9.10
50	9.20	10.80	14.96
100	11.87	15.48	20.10
200	16.15	23.13	24.52

A well polished gallbladder stone was placed on the platform of Vickers micro hardness tester and loads of different magnitude (25, 50, 100 and 200 gm) was applied in a fixed interval of time. The indentation time was kept as 15 seconds for all the loads.

A graph was plotted between hardness values and applied load as shown in Figure 3. Beyond the load of 200 gms, a significant cracking occurs which may be due to the release of internal stresses generated by indentation. Higher the hardness value, greater the stress required to form dislocation, thus conforming greater crystalline perfection. From the Table 2, it is observed that Mixed stone category have higher hardness value indicating higher resistance to plastic deformation and proving that greater the stress required to form dislocation, better is its crystalline nature.

The result was also confirmed by Ultrasonic velocity and Debye's temperature measurement. The Bilirubinate stone is a moderately hard material than a Cholesterol stone. From the Figure 3, it is clear that the hardness value of Cholesterol stones (White and Whitish brown), Bilirubinate stones (Brown) and Mixed stone (Dark Brown and Black) increases and then attains almost saturation with the increase in the applied load.

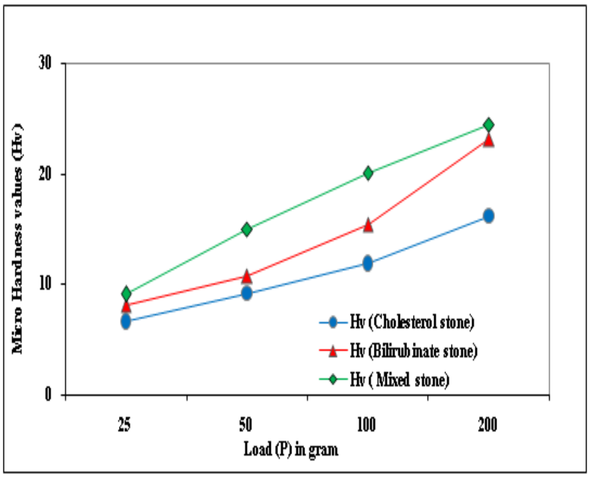

Figure 3: Trend Graph of Micro Hardness Values (H_v) of Cholesterol (White, Whitish Brown), Bilirubinate (Brown) and Mixed (Dark Brown, Black) Gallbladder Stones

The value of Hardening coefficient (n) was estimated from the plot of logarithmic scales of load (P) v/s logarithmic mean diagonal length (d) of Cholesterol stones (White and Whitish Brown), Bilirubinate stones (Brown) and Mixed stone (Dark Brown and Black) respectively by the least square fit method which is shown in Figure 4.

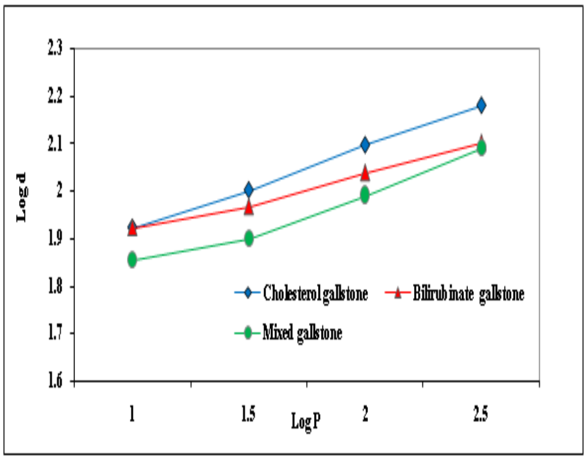

Figure 4: Trend Graph of Hardness Coefficient (n) of Cholesterol (White, Whitish Brown) Bilirubinate (Brown) and Mixed (Dark Brown, Black) Gallbladder Stones

The mean Hardening coefficient (n) of gallstones was calculated and presented in Table 3 and is useful to check whether the material is hard or soft. As the Vickers hardness test results are in correlation with the Ultrasonic results and also, as the Hardening coefficient value (n) for all the three type of gallbladder stones are greater than 1.6 [24, 25], we can conclude that gallbladder stones are soft materials and they differ only by hardness. The variation in hardness is mainly related to the chemical composition of the gallbladder stones [26].

Table 3: Hardening Coefficient (n) of Gallbladder Stones

Types of Gallbladder Stones	Hardening Coefficient (n)
Cholesterol stones (White & Whitish Brown stones)	3.75 ± 0.25 (3.32– 4.20)
Bilirubinate stones (Brown stones)	4.87 ± 0.38 (4.44 – 5.15)
Mixed stones (Dark Brown & Black stones)	3.30 ± 0.08 (3.24 – 3.44)

Figure 5 shows the mean value score of the three types of gallbladder stones and their relative distribution of hardness.

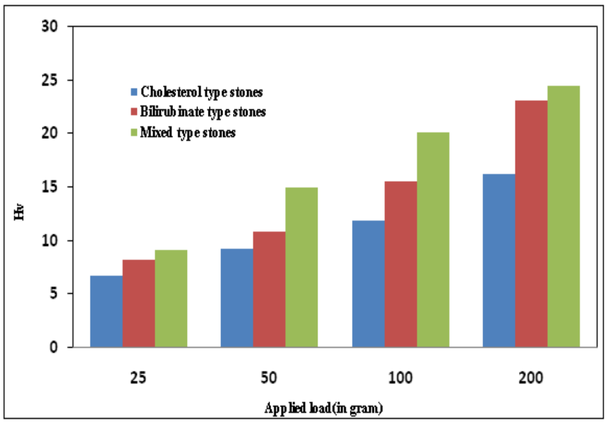

Figure 5: The Relative Distribution of Hardness (H_v) of Gallbladder Stones with Various Loads

The bar diagram of mean hardening coefficient (n) of the three types of gallbladder stones is shown in Figure 6.

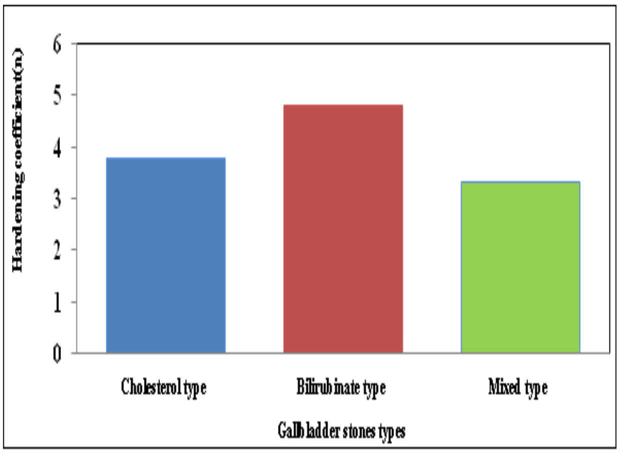

Figure 6: The Relative Distribution of Hardening Coefficient (n) of Gallbladder Stones

Elastic Stiffness Constant

The Elastic Stiffness constant (C_{11}) is also calculated using Wooster's empirical formula which gives an idea about the strength and tightness of bonding between the neighbouring atoms and the results are discussed. From the mean value of Elastic Stiffness constant (C_{11}) presented in Table 4, it is clear that among the types of gallbladder stones, Cholesterol stones have a lower Elastic Stiffness constant whereas, Mixed stones have a higher Elastic Stiffness constant than Bilirubinate stones. This variation is mainly due to the chemical composition of the gallbladder stones. Also, an increase in the Elastic stiffness constant (C_{11}) value is due to the increase in strength and tightness of the molecular binding on increasing the applied load.

Table 4: Elastic Stiffness Constant (C_{11}) Values (in GPa) for the Three Types of Gallbladder Stones

Applied Load (in Gram)	Cholesterol Stones (White & Whitish Brown Stone)	Bilirubinate Stones (Brown Stone)	Mixed Stones (Dark Brown & Black Stone)
25	0.27	0.39	0.47
50	0.48	0.63	1.11
100	0.74	1.18	1.90
200	1.27	2.40	2.65

Figure 7 shows the mean values score of three types of gallbladder stones and the relative distribution of Elastic stiffness (C_{11}).

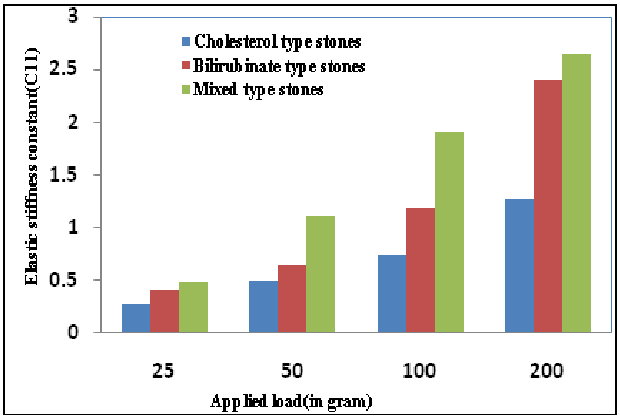

Figure 7: The Relative Distribution of Elastic Stiffness (C_{11}) of Gallbladder Stones with Various Loads

Statistical Analysis

Statistical analyses provide an objective appraisal of experimental results instead of relying on the subjective impression. Here, the parameters estimated from gallbladder stone samples are used to calculate the statistical data. Table 5 shows the statistical analysis of all the three types of gallbladder stones samples. The data's were statistically analyzed using the technique of one way Analysis of Variance (ANOVA). A test of significance is applied on Vickers micro hardness values of gallbladder stones as shown in Table 5. Using Vickers micro hardness techniques, the problem is investigated by comparing the hardness of gallbladder stones samples. The mean value, standard deviation of hardness and ANOVA results are given in Table 5 for all the three types of stones at $p<0.01$ significant level for various loads (25, 50, 100 and 200 gram).

Table 5: Mean and Standard Deviation of Hardness Values for All the Three Types of Gallbladder Stones by Vickers Hardness Test Method – Statistical Analysis

Applied Load (in Gram)	Cholesterol Stones (White & Whitish Brown Stones)		Bilirubinate Stones (Brown Stones)		Mixed Stones (Dark Brown & Black Stones)		*F* Value	*p* Value
	Mean	SD	Mean	SD	Mean	SD		
25	6.63	0.83	8.16	0.56	9.10	0.51	18.51	0.0000^{s}
50	9.20	1.23	10.80	1.77	14.96	0.74	25.51	0.0000^{s}
100	11.87	0.81	15.48	0.90	20.10	0.55	144.18	0.0000^{s}
200	16.15	0.35	23.13	0.56	24.52	0.41	479.27	0.0000^{s}

(S-Significant)

From the Statistical analysis as shown in Table 5, it is clear that among the three types of gallbladder stones, the presence of cholesterol contributes to the lesser value of hardness in the Cholesterol stone (white and whitish brown).

Brown stones formed by the super-saturation of bile, have higher inorganic compounds that are responsible for the moderate hardness in Bilirubinate stones and the Mixed stone (Dark Brown and Black stones) show higher value towards hardness, mainly due to the maximum calcium contents [27].

CONCLUSIONS

The values of ultrasonic velocity and other derived acoustical parameter have been measured for various types of gallbladder stones. The data may be useful for design of an ultrasonic disintegrator.

Micro hardness study was also carried out for these types of gallbladder stones and it varies non-linearly with composition. The Elastic stiffness constant for these gallbladder stones was also measured which gives an idea about the strength and tightness of molecular binding within the stone.

The statistical analysis of Vickers hardness measurements was carried out in order to check the reliability of hardness measurements.

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