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Ultrasonic Relaxation and Spectroscopy Studies on Gallstones

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Abstract: ***Bile salts are naturally occurring biological surfactants. They form micellar aggregates in aqueous solutions at a particular bile salt concentration called Critical Micelle Concentration (CMC). These micellar aggregates help to solubilise and disperse dietary lipids in small intestine. It is believed that the micelles formed by bile salts play a vital role in the early crystallization which in turn leads to gall stone formation in human beings. The present ultrasonic relaxation studies have been carried out on some gallstones at a fixed temperature of 303.15K and in the frequency range 2-15 MHz in order to ascertain the relaxation frequency and relaxation amplitudes respectively. The ultrasonic velocity is determined at 2 MHz using a Digital Ultrasonic velocity meter. The ultrasonic absorption is measured in the frequency range of 2-15 MHz using Fallen Instruments and Pulsed Power Oscillator systems. The relaxation frequency (f_r) and relaxation parameters (A&B) are determined and reported. Fourier transform infrared spectroscopy study is used to determine the constituents and their compositions of gallstones. The study reveals that cholesterol stones and mixed stones type of gallstones were predominant whereas pigment stones were less frequent in the selected region.***

Keywords: Ultrasonic velocity, ultrasonic absorption, micellar aggregates, Gallstones

I. INTRODUCTION

Gallstones are solid particles that form from bile in the gallbladder, which is a small saclike organ in the upper right part of the abdomen. It is located under the liver, just below the front rib case on the right side. The gallbladder is a part of the biliary system, which includes the liver and the pancreas. The biliary system, among other functions, produces bile and digestive enzymes. Bile is a fluid secreted by the liver to help in the digestion of fats [1]. It contains several different substances, including cholesterol and bilirubin a waste product of normal breakdown of blood cells in the liver. Bile is stored in the gallbladder until needed. When we eat a high – fat, high – cholesterol meal, the gallbladder contracts and injects bile into the small intestine via a small tube called the common bile duct for digestion [2]. Gallstones form in the gallbladder because the liver begins secreting bile that is unusually saturated with cholesterol. The cholesterol then crystallizes to form stones while in storage in the gallbladder or cystic duct. The specific mechanisms in the body which cause the bile to reach these high cholesterol levels are not entirely known. However, the liver appears to play a large part in this process since it is the source for making bile from which gallstones can form because the amount of bile acids and bile lecithin are low. Bile acids and lecithin act as an emulsifier in the bile within the gallbladder. Therefore, any inefficiency in this emulsifying process would accelerate the formation of stones. From a survey of literature, it can be seen that most of the ultrasonic relaxation studies in surfactants are confined only to aqueous surfactants [3-9], surfactant dissolved in aqueous-alcoholic [10-12] and surfactant dissolved in aqueous-electrolytes [13]. In addition gallstones, however, a number of different techniques such as X-ray diffraction studies [14-17], FTIR studies [18-19], and Micro-elemental studies [20-22]. From a survey of literature, ultrasonic relaxation studies on gallstones are scandy. Moreover, our earlier ultrasonic relaxation studies in aqueous solution of sodium taurocholate and sodium cholate, sodium deoxycholate revealed interesting results [23-24]. Hence, the present relaxation study has been undertaken in order to report the relaxation frequency and the relaxation amplitudes for different types of gallstones.

II. MATERIALS AND METHOD

The gallstones used in the present study are collected from Jawaharlal Institute of Post Graduate Medical Education and Research (JIPMER) Pondicherry, India. All the stone are washed in running water and air dried for several days. The collected gall stones were powdered using clean pestle and agate mortar. The powered samples are then pelletised by applying 15-16 KPa pressure. The ultrasonic velocity and absorption measurements are carried out for various gallstone samples at a fixed temperature of 303.15 K. The ultrasonic velocity is measured at an RF frequency of 2 MHz using a Digital Ultrasonic velocity meter (Model VCT-70A, Vi-Microsystems Private Ltd., Chennai, India). The ultrasonic absorption is measured in the frequency range of 2-15 MHz using Fallen

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Instruments and Pulsed Power Oscillator systems. FTIR studies were carried out using NICOLET 6700 FTIR SYSTEM at IIT Chennai, India. During the FTIR studies the frequency range 4000–400 cm^{-1} at 4 cm^{-1} resolutions is used. To obtain a high signal/noise ratio 100 scans were accumulated for each sample. This study is useful to generate a detailed collection of data on different types of gallstones formation among the peoples of Pondicherry and the southern region of Tamil Nadu.

III. RESULTS AND DISCUSSION

A. Ultrasonic Relaxation Studies

The variations of observed absorption of gallstone samples at different frequencies are shown in the figures 1-7. The observed absorption data obtained for the gallstones are fitted to the following Debye type equation (1) for single relaxation using a non-linear least square fitting program based on Marquardt method [25].

$$\left(\frac{\alpha}{f^2}\right)_{obs} = \frac{A}{1+\left(\frac{f}{f_r}\right)^2} + B \qquad (1)$$

(or)

$$(\alpha\lambda) = Afu\left[1+\left(\frac{f}{f_r}\right)^2\right] \qquad (2)$$

In the above equations (1) and (2), $(\alpha/f^2)_{obs}$ is the observed ultrasonic absorption, f represents the experimental frequency, f_r is the relaxation frequency, A is the relaxation amplitude, B is the contribution to sound absorption from any other processes that may be occurring at higher frequencies beyond our frequency range and u is the ultrasonic velocity. Using the values of A, B and f_r, experimental absorption per wavelength $(\alpha\lambda)_{exp}$ and calculated absorption per wavelength $(\alpha\lambda)_{cal}$ were obtained for all the gallstones studied.

The variations of absorption per wavelength ($\alpha\lambda$) with frequency of the gallstone samples in the frequency range studied are shown in figures 7-14. The solid lines in the figures 1-7 represent the calculated ultrasonic spectra from equations (1) and the solid lines in the figures 8-14 represent the calculated ultrasonic spectra from equations (2) and the arrows in figures 1-14 indicate the location of relaxation frequency. From the figures 1-14, it can be observed that the experimental data fit well to the equations (1) and (2), thus showing that all types of the gallstones show single relaxation process. Tables 1-2 summarises the relaxation parameters obtained for gallstone samples. From the figures 1-7, it can be seen that observed ultrasonic absorption decreases with increase in frequency. Whereas from the figures 8-14, its can be seen that absorption per wavelength ($\alpha\lambda$) increases with increase of frequency reaching a maximum. With further increase in frequency absorption per wavelength ($\alpha\lambda$) decreases. From the figures (1-14), it can be shown that different types of gallstones shows different values of relaxation frequency. The observed cholesterol gallstone relaxation frequency range (3.82-3.89), mixed gallstone relaxation frequency (3.18-3.25) and pigment gallstone relaxation frequency range (3.69). This difference in relaxation frequency may be due to the different composition of the gallstone samples.

B. FTIR Studies

FTIR spectrometer (NICOLET 6700 FT - IR) is used to identify the functional groups and measurements are carried out in the mid - infrared range (4000 - 400 cm^{-1}) at 4 cm^{-1} resolution rate in the transmittance mode. FTIR spectra of 7 samples are shown in figures 15 – 21. The chemical components and its corresponding IR transmittance bands of gallstones are given under the FTIR spectra. From the FTIR spectra, the collected gallstones were grouped into cholesterol (4), mixed (2) and pigment (1) type gallstones. This analysis showed that cholesterol crystal is the predominant composition in cholesterol and mixed gallstones.

1) *Cholesterol Stones:* Presence of cholesterol in the gallstone samples GS 2, GS 3, GS 4 and GS 7 is characterized by large O – H stretching absorption bands at 3394.0, 3398.3 cm^{-1}, C - H stretching vibration band occurring from 2935.4 to 2941.3 cm^{-1}, C – H deformation bands obtained at 1463.8, 1461.9 cm^{-1} in the FTIR spectrum of these samples. A sharp absorption peak is observed at 1054.9, 1053.09 cm^{-1} due to the ring deformation of cholesterol [26-28]. Band occurring from 1373.3 to 1378.2 cm^{-1} is due to CH_2 and CH_3 bending vibration of cholesterol gallstones [27-28, 29-31]. A very weak intensity band at 1666.3 cm^{-1}

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due to bilirubin salts. Aragonite form of $CaCo_3$ shows weak intensity band at from 1085 and 699 cm^{-1} [32]. The presence of calcium is identified by the C – O stretching bands occurring between1461.9 - 1463.9 cm^{-1}.

2) *Mixed Stones:* In GS 1and GS 6 cholesterol is abundant as described in cholesterol stones from GS 2, 3, 4, 7. Bilirubinate salts have characteristic bands at 1645.8 cm^{-1} and a band at 3442.6 cm^{-1} due to N –H stretching vibration of pyrrole of bilirubin, the 1647.1 cm^{-1} band also comes from bilirubinate salt. Generally, calcium palmitate is the most abundant component of gallstone. *Identification of calcium palmitate could be based on the presence of specific peaks at 1461 and 668.8 cm^{-1}.*

3) *Pigment Stones:* In FTIR of GS 5 is identified to have as calcium bilirubinate salts. This must be the reason for the amorphous nature of these gallstones. Calcium bilirubinate have characteristic band at 1615.6, 1622,1246.2, 1666.4, 1453.5, 1571.3 cm^{-1} which are assigned to (C = C, C – N, C = O) stretching vibration of lactam, C = O stretching of COOH, (C = O ,C – N, C = C) stretching, asymmetric stretching γ as (COO) and (C – O) stretching or C – N stretching coupled with NH deformation γ (C –N) + δ (NH), respectively [31, 33-35] Calcium palmitate could be identified by the presence of specific peaks at 612.5, 856.2, 2921.9 and 2923.8 cm^{-1}. Weak O – H stretching absorption band at 3398.3, 3352.0 cm^{-1} and sharp absorption peak at 1041.4, 1043.4 cm^{-1} can be attributed to the ring deformation of cholesterol.

FTIR analysis of gallstones shows that cholesterol to be the most abundant component, bilirubin is the next abundant component. Calcium carbonate ($CaCo_3$) in the form of two polymorphs namely calcite and aragonite obtained by FTIR studies.

IV. CONCLUSION

The ultrasonic relaxation studies carried out for all the gallstone samples which show the observed absorption decreases with increasing of frequency. The observed absorption variation follows a single relaxation process. The possible relaxation mechanism in the present study is due to the exchange of energy from chemical composition of gallstone samples. The different gallstones have different relaxation frequency due to the different chemical composition presented within the gallstone samples. Seven stones were analysed by Fourier Transform Infrared Spectroscopy (FTIR). From these studies it is found that cholesterol was the main component in GS 1, GS 2, GS 3, GS 4, GS 6, and GS 7, remaining stone (GS 5) is found to be pigment calcium stones by FTIR studies. It has been identified that in mixed stones (GS 1 and GS 6) cholesterol also is found to be abundant. Next to cholesterol derivatives bilirubin occurrence is more, this is due to the presence of bilirubin in cholesterol stones and calcium bilirubinate in pigment calcium gallstones. These results are confirmed by FTIR studies.

From the ultrasonic relaxation and FTIR studies finally concluded that the majority of patients are affected by cholesterol and mixed gallstones compared to pigment gallstones. These cholesterol stones formations may be due to the intake of food, age factor and liver diseases in and around the region of Pondicherry peoples.

V. ACKNOWLEDGEMENT:

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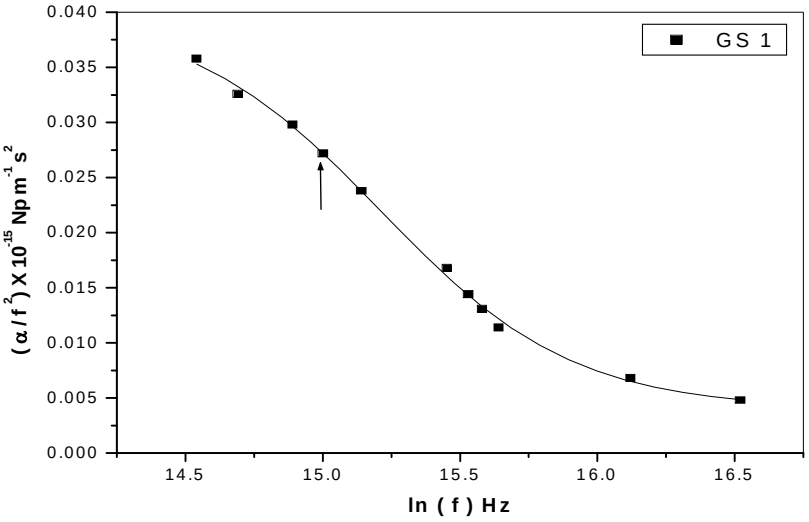


Figure -1 Plot of observed absorption (α/f^2)$_{obs}$ against ln (f) for GS 1.

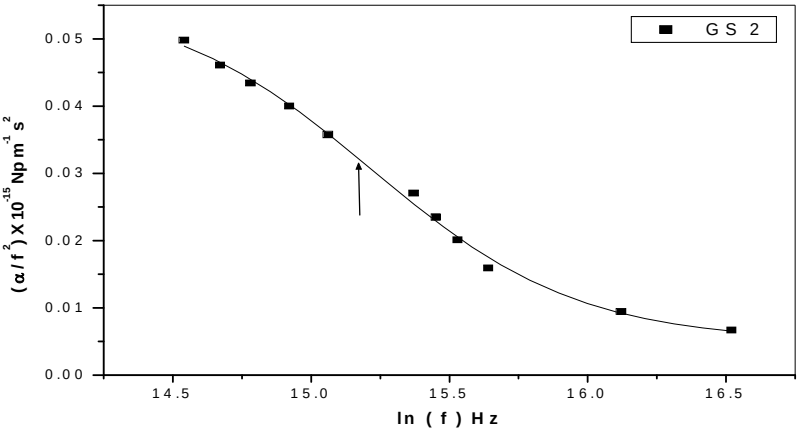


Figure - 2 Plot of observed absorption (α/f^2)$_{obs}$ against ln (f) for GS 2.

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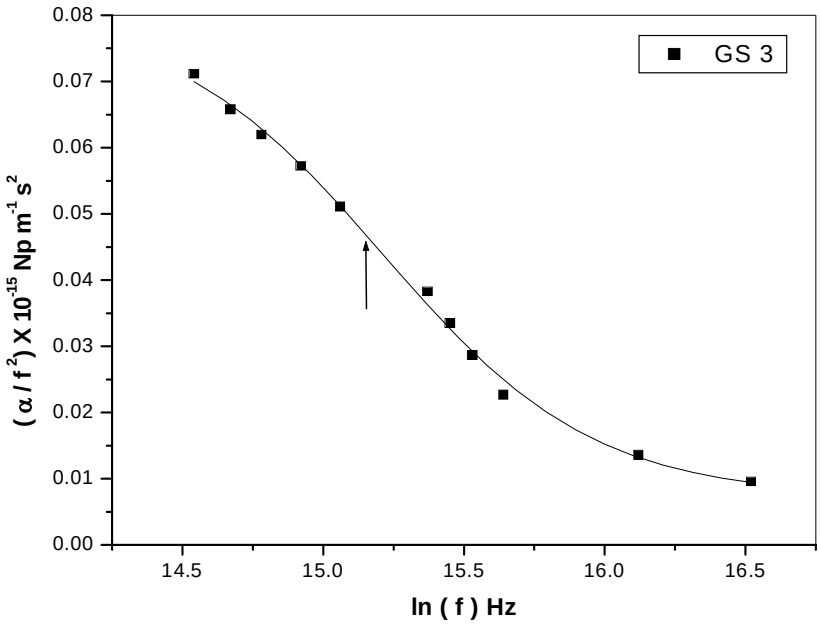


Figure -3 Plot of observed absorption $(\alpha/f^2)_{obs}$ against ln (f) for GS 3.

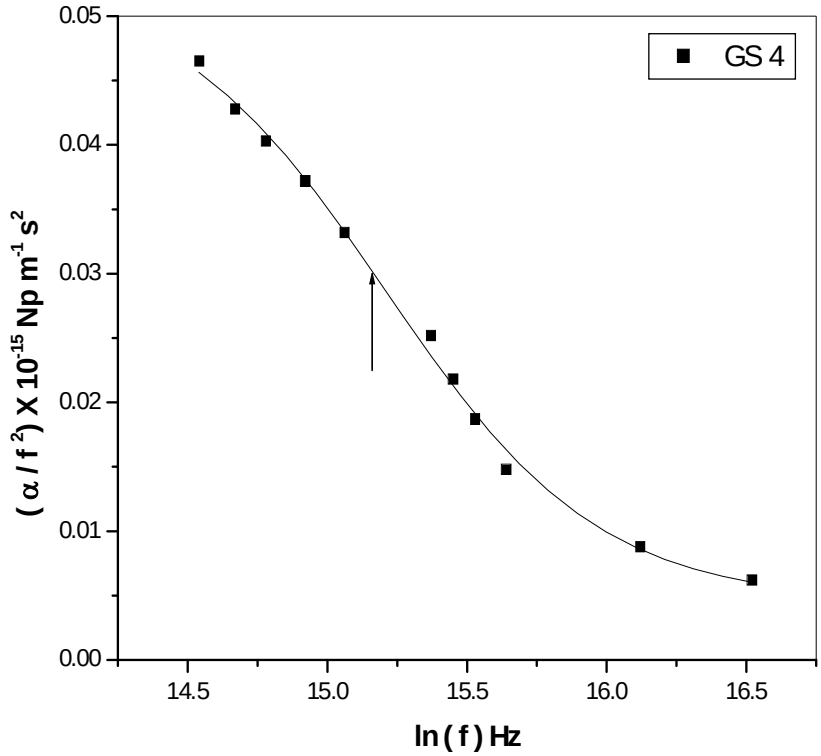


Figure - 4 Plot of observed absorption $(\alpha/f^2)_{obs}$ against ln (f) for GS 4.

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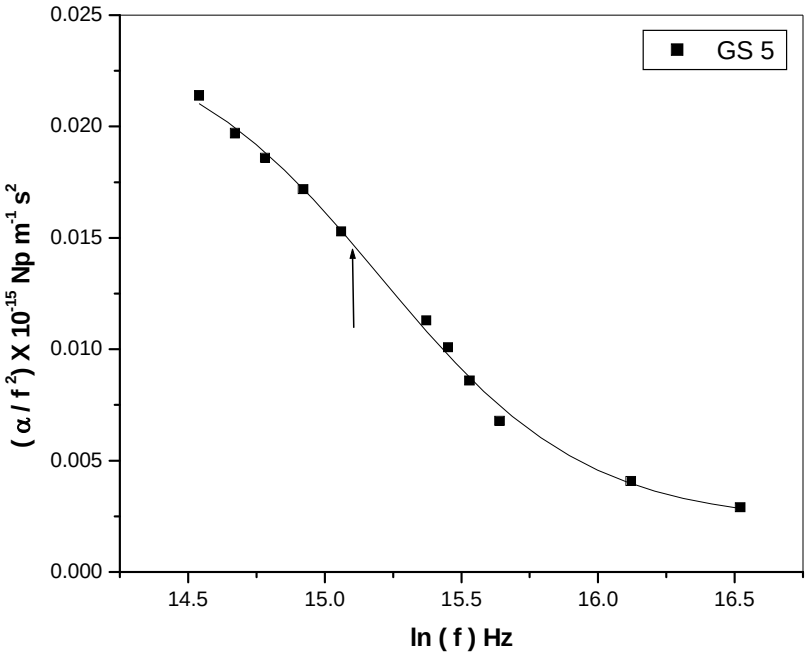


Figure -5 Plot of observed absorption $(\alpha/f^2)_{obs}$ against ln (f) for GS 5.

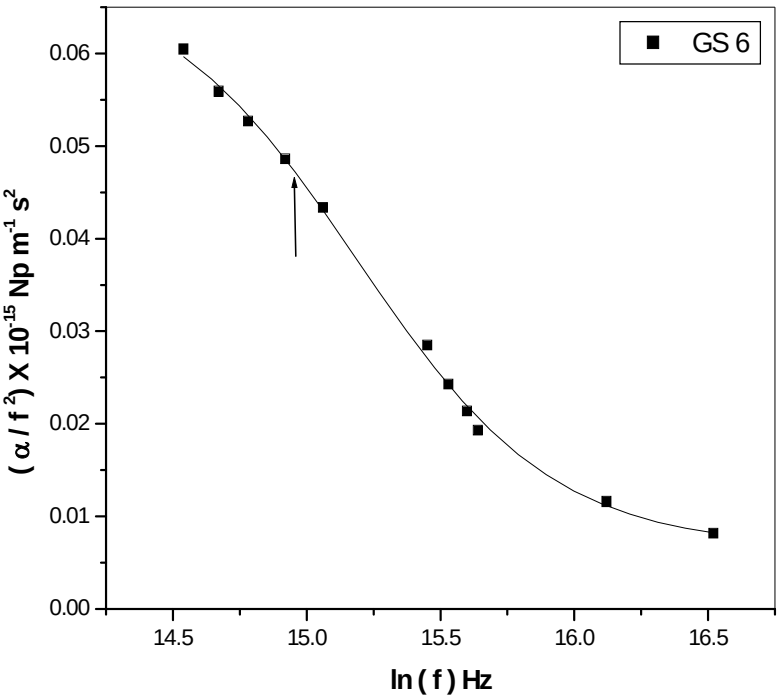


Figure - 6 Plot of observed absorption $(\alpha/f^2)_{obs}$ against ln (f) for GS 6.

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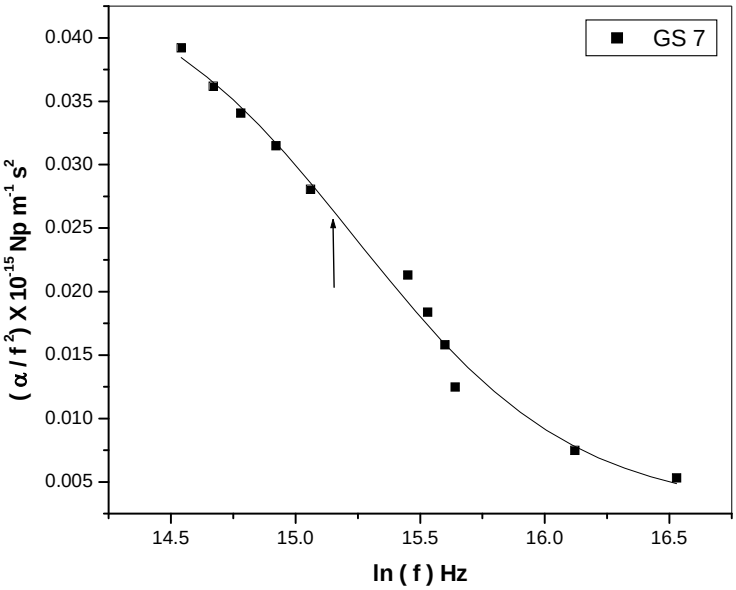


Figure - 7 Plot of observed absorption $(\alpha/f^2)_{obs}$ against ln (f) for GS 7.

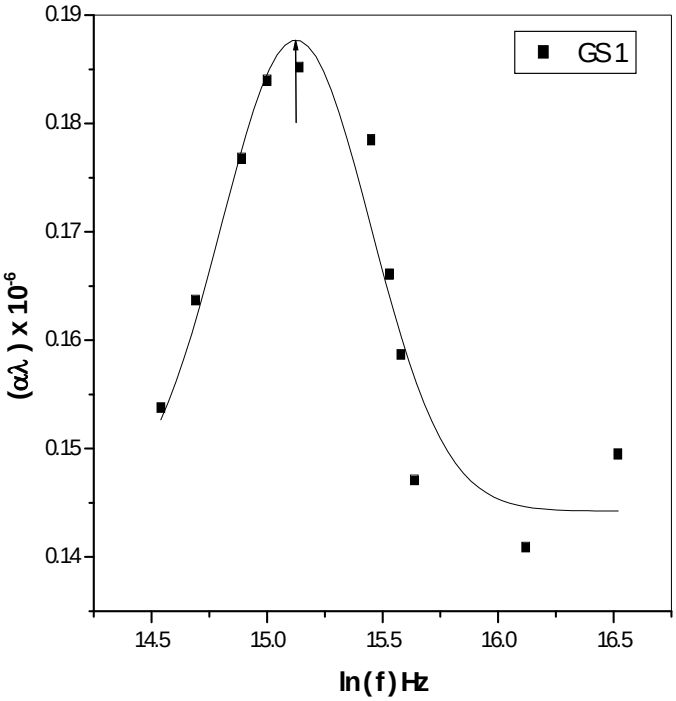


Figure -8 Plot of absorption per wavelength (αλ) against ln (f) for GS 1.

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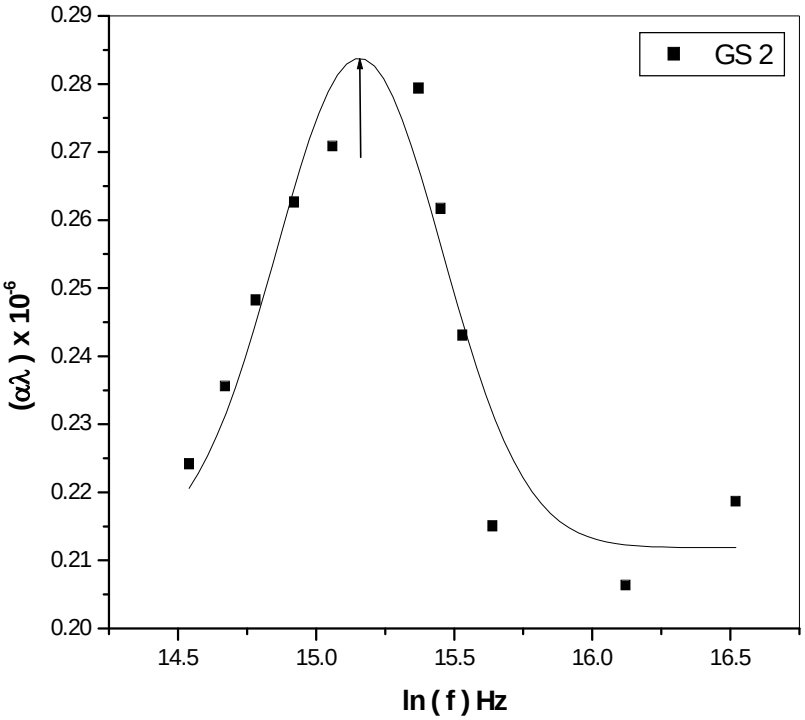


Figure - 9 Plot of absorption per wavelength (αλ) against ln (f) for GS 2.

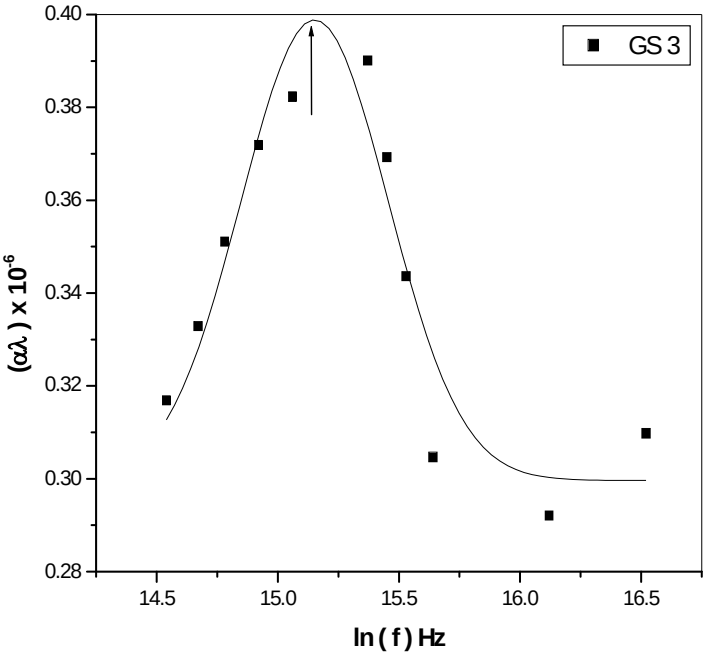


Figure -10 Plot of absorption per wavelength (αλ) against ln (f) for GS 3.

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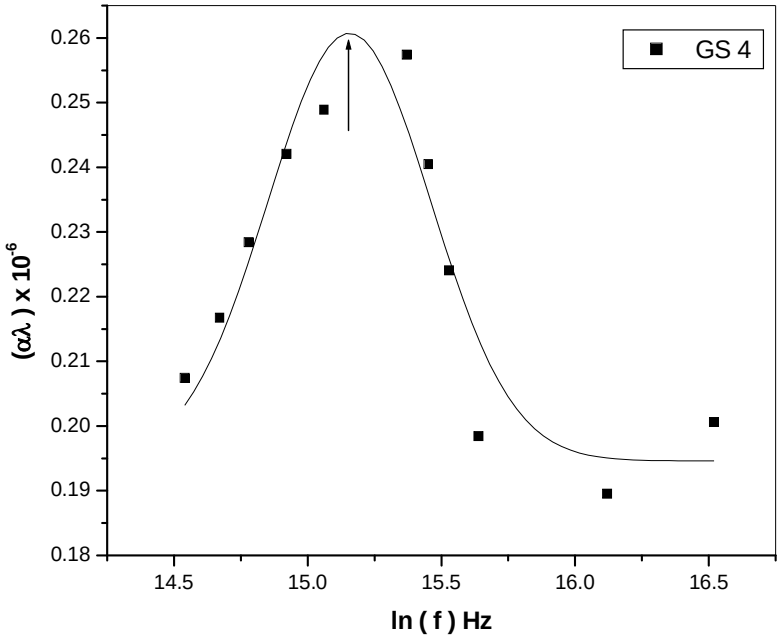


Figure - 11 Plot of absorption per wavelength (αλ) against ln (f) for GS 4

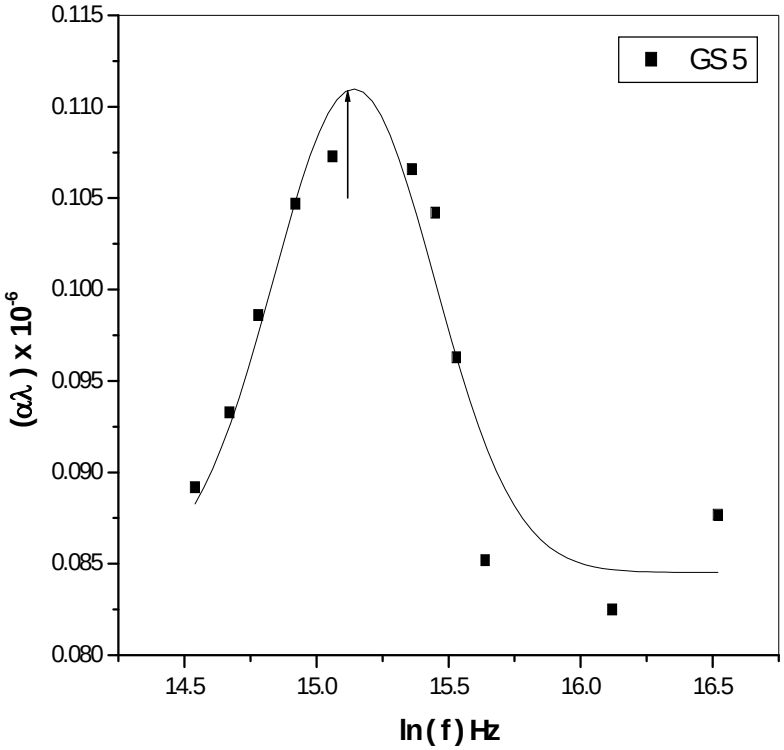


Figure -12 Plot of absorption per wavelength (αλ) against ln (f) for GS 5

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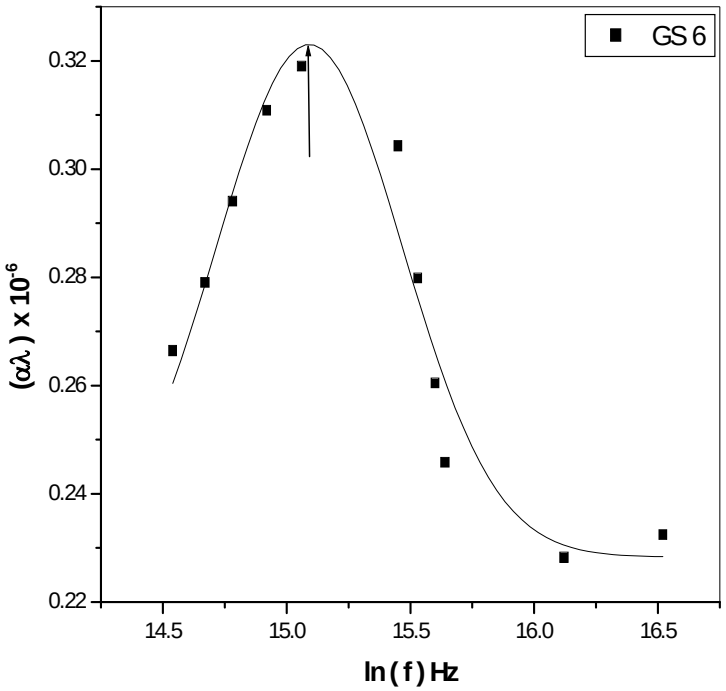


Figure - 13 Plot of absorption per wavelength (αλ) against ln (f) for GS 6

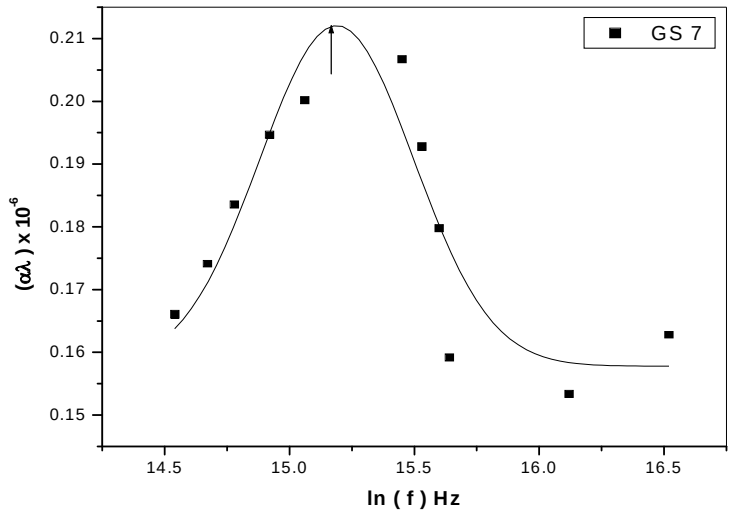


Figure - 14 Plot of absorption per wavelength (αλ) against ln (f) for GS 7

Table 1
Ultrasonic velocity, observed absorption, Observed absorption per wave length, observed, calculated absorption per wave length maxima, relaxation frequency and relaxation amplitudes for different gallstone samples

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Sample Number	u $m\ s^{-1}$	f MHz	$(\alpha / f^2)_{obs}$ 10^{-15} $Np\ m^{-1}\ s^2$	$(\alpha\lambda)_{obs}$ 10^{-6}	Calculated $(\alpha\lambda)_{max}$ 10^{-6}	Observed $(\alpha\lambda)_{max}$ 10^{-6}	f_r MHz	A 10^{-15} $Np\ m^{-1}\ s^2$	B 10^{-15} $Np\ m^{-1}\ s^2$
GS 1	2075	2.07 2.42 2.86 3.26 3.75 5.12 5.56 5.84 6.22 9.99 15.01	0.0358 0.0326 0.0298 0.0272 0.0238 0.0168 0.0144 0.0131 0.0114 0.0068 0.0048	0.1538 0.1637 0.1768 0.1840 0.1852 0.1785 0.1661 0.1587 0.1471 0.1409 0.1495	0.1895	0.1873	3.2482	0.0563	0.0089
GS 2	2175	2.07 2.35 2.63 3.02 3.48 4.74 5.12 5.56 6.22 9.99 15.01	0.0498 0.0461 0.0434 0.0400 0.0358 0.0271 0.0235 0.0201 0.0159 0.0095 0.0067	0.2242 0.2356 0.2483 0.2627 0.2710 0.2794 0.2617 0.2431 0.2151 0.2064 0.2187	0.3581	0.2846	3.8934	0.0884	0.0017
GS 3	2015	2.07 2.35 2.63 3.02 3.48 4.74 5.12 5.56 6.22 9.99 15.01	0.0712 0.0658 0.0620 0.0572 0.0511 0.0387 0.0335 0.0287 0.0227 0.0136 0.0096	0.3169 0.3329 0.3511 0.3719 0.3823 0.3901 0.3693 0.3437 0.3047 0.2921 0.3098	0.4579	0.3988	3.8262	0.1188	0.0052
GS 4	2155	2.07 2.35 2.63 3.02 3.48 4.74 5.12 5.56 6.22 9.99 15.01	0.0465 0.0428 0.0403 0.0372 0.0332 0.0252 0.0218 0.0187 0.0148 0.0088 0.0062	0.2074 0.2168 0.2284 0.2421 0.2489 0.2574 0.2405 0.2241 0.1984 0.1895 0.2006	0.3267	0.2615	3.8880	0.0779	0.0009

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Table 2

Ultrasonic velocity, observed absorption, Observed absorption per wave length, observed, calculated absorption per wave length maxima, relaxation frequency and relaxation amplitudes for different gallstone samples

Sample Number	u $m\ s^{-1}$	f MHz	$(\alpha / f^2)_{obs}$ 10^{-15} Np m^{-1} s^2	$(\alpha\lambda)_{obs}$ 10^{-6}	Calculated $(\alpha\lambda)_{max}$ 10^{-6}	Observed $(\alpha\lambda)_{max}$ 10^{-6}	f_r MHz	A 10^{-15} Np m^{-1} s^2	B 10^{-15} Np m^{-1} s^2
GS 5	2015	2.07 2.35 2.63 3.02 3.48 4.69 5.12 5.56 6.22 9.99 15.01	0.0214 0.0197 0.0186 0.0172 0.0153 0.0113 0.0101 0.0086 0.0068 0.0041 0.0029	0.0893 0.0933 0.0986 0.1047 0.1073 0.1066 0.1042 0.0964 0.0852 0.0825 0.0877	0.1232	0.1113	3.6962	0.0331	0.0030
GS 6	2165	2.07 2.35 2.63 3.02 3.48 5.12 5.56 5.91 6.22 9.99 15.01	0.0605 0.0559 0.0527 0.0486 0.0434 0.0285 0.0243 0.0214 0.0193 0.0116 0.0082	0.2711 0.2844 0.3001 0.3178 0.3269 0.3159 0.2925 0.2734 0.2598 0.2509 0.2665	0.3357	0.3238	3.1764	0.0976	0.0218
GS 7	2047	2.07 2.35 2.63 3.02 3.48 5.12 5.56 5.96 6.22 9.99 15.01	0.0392 0.0362 0.0341 0.0315 0.0281 0.0213 0.0184 0.0154 0.0125 0.0075 0.0053	0.1661 0.1741 0.1836 0.1947 0.2002 0.2067 0.1928 0.1798 0.1592 0.1534 0.1628	0.2458	0.2125	3.8516	0.0623	0.0027

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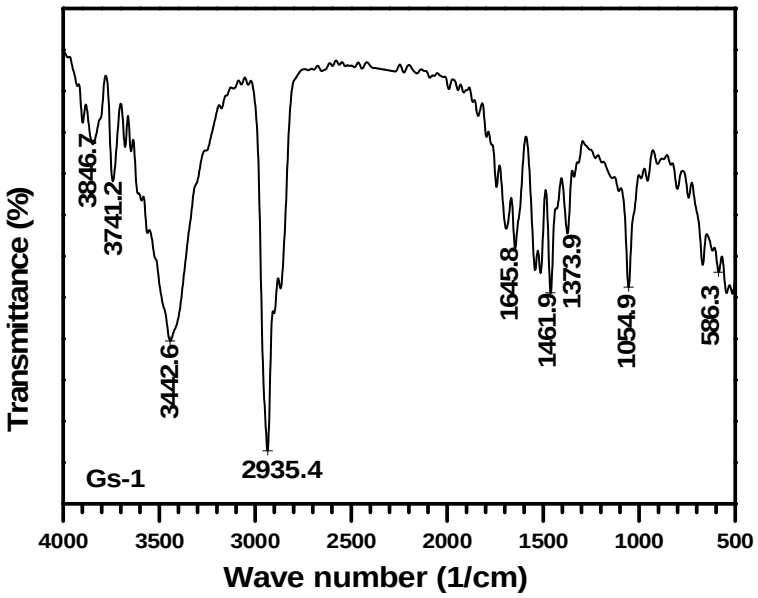


Figure – 15: FTIR Spectrum of GS 1

Wave number cm^{-1}	Compound
3846.7	Aragonite
3442.6	Aragonite
2935.4	Cholesterol
1645.8	Calcium palmitate
1461.9	Cholesterol
1373.9	Cholesterol
1054.9	Bilirubin
586.3	Calcium phosphate

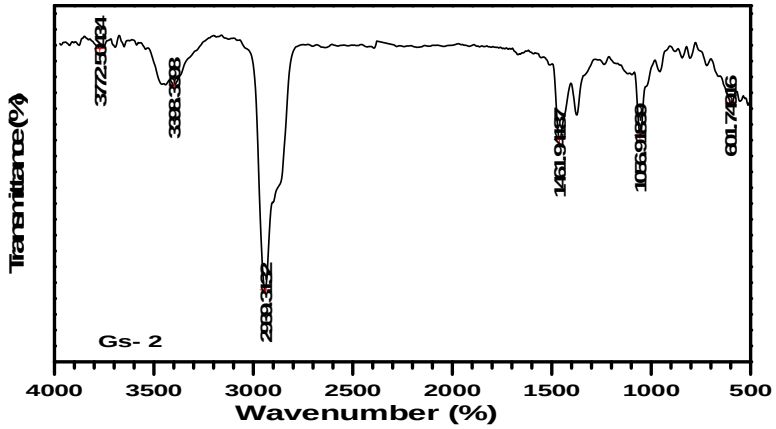


Figure – 16: FTIR Spectrum of GS 2

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Wave number cm^{-1}	Compound
3398.3	Cholesterol
2939.3	Calcium palmitate / Cholesterol
1461.9	Cholesterol
1056.9	Cholesterol / Bilirubin
601.7	Cholesterol

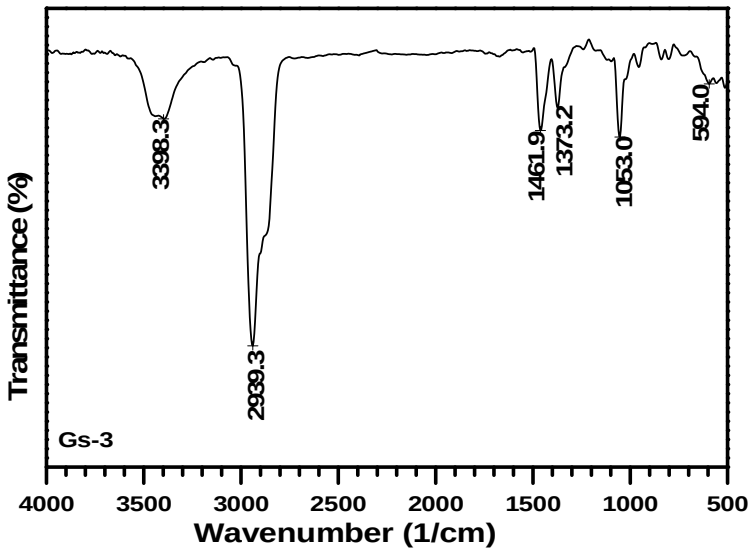


Figure – 17: FTIR Spectrum of GS 3

Wave number cm^{-1}	Compound
3398.3	Cholesterol
2939.3	Calcium palmitate / Cholesterol
1461.9	
1373.2	Cholesterol
1053.0	Cholesterol
594.0	Cholesterol / Bilirubin
	Cholesterol

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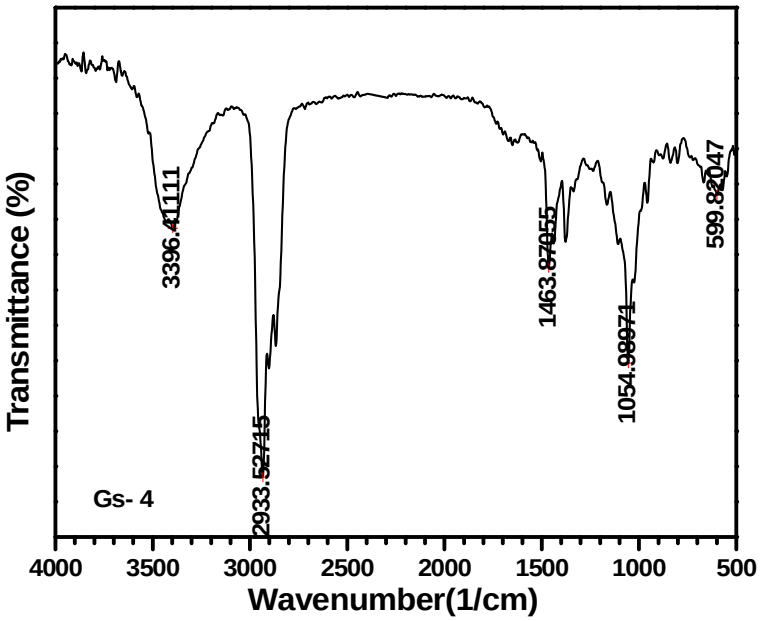


Figure – 18: FTIR Spectrum of GS 4

Wave number cm^{-1}	Compound
3396.4	Bilirubin/ Cholesterol
2933.5	Cholesterol
1463.9	Cholesterol / calcium palmitate
1054.9	Cholesterol / Bilirubin
599.8	Cholesterol

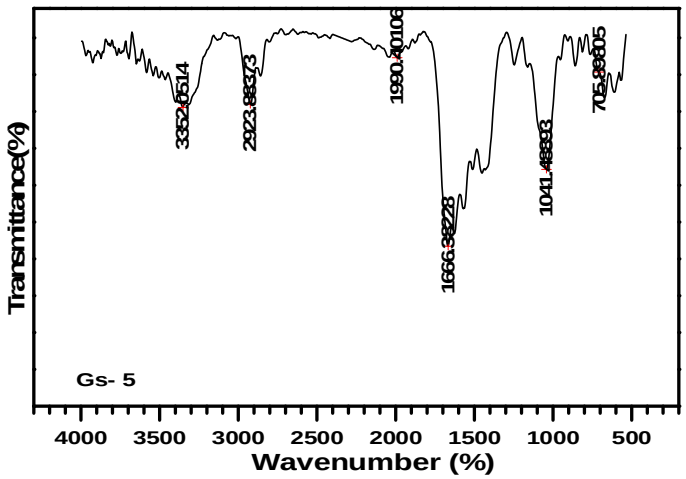


Figure – 19: FTIR Spectrum of GS 5

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Wave number cm^{-1}	Compound
3352.0	Bilirubin
2923.8	Cholesterol / Calcium palmitate
1666.3	Calcium palmitate
1041.5	Bilirubin /Cholesterol
705.9	Calcium carbonate/Araganite

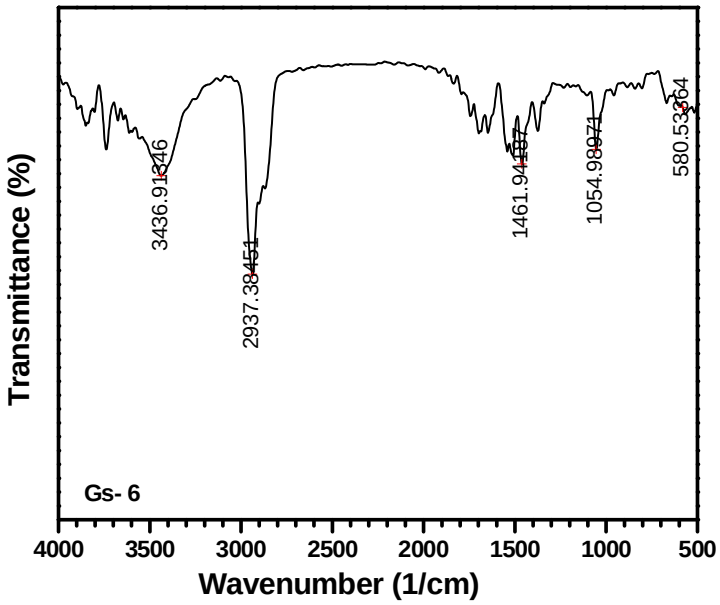


Figure – 20: FTIR Spectrum of GS 6

Wave number cm^{-1}	Compound
3436.9	Aragonite
2937.3	Cholesterol / Calcium palmitate
1461.9	Aragonite
1054.9	Bilirubin / Cholesterol
580.5	Calcium palmitate / Calcium phosphate

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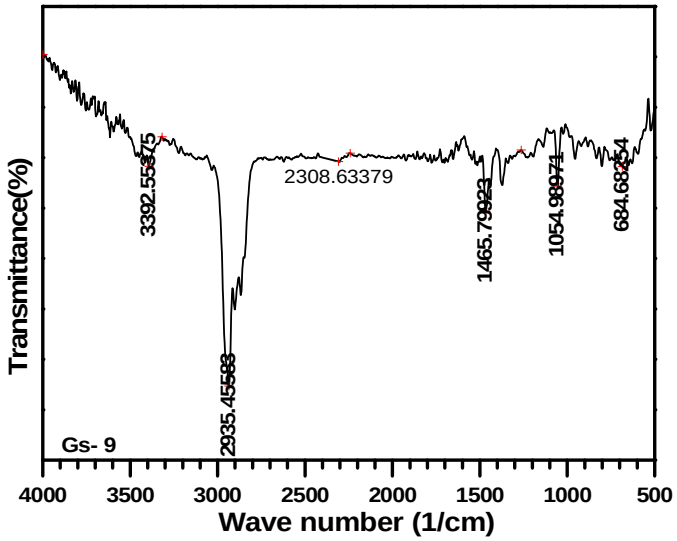
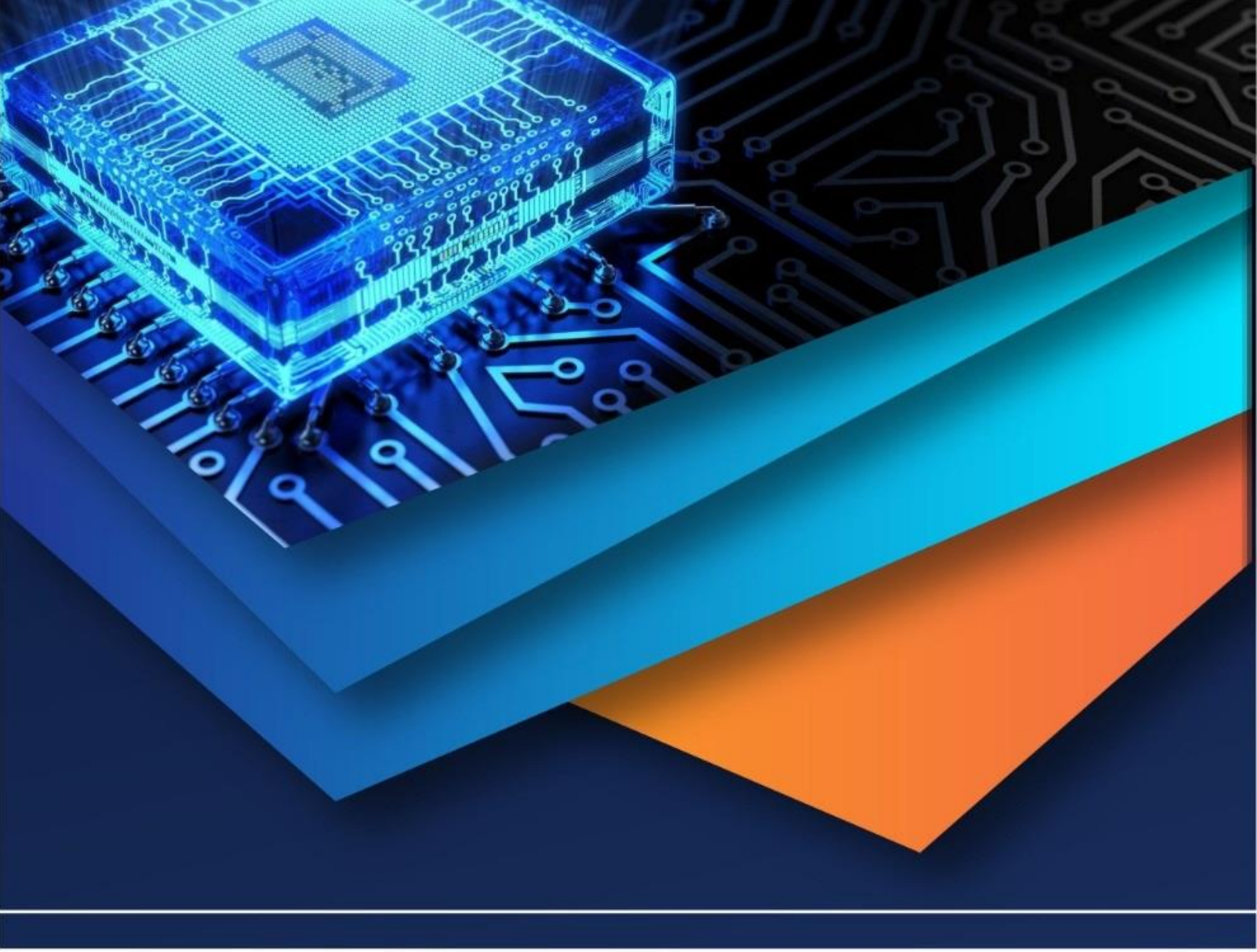


Figure – 21: FTIR Spectrum of GS 7

Wave number cm^{-1}	Compound
3392.5	Bilirubin/ Cholesterol
2935.5	Cholesterol
1465.8	Cholesterol / Calcium palmitate
1054.9	Cholesterol
684.7	Araganite

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