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INVESTIGATIONS ON THE GROWTH OF URINARY STONE CRYSTALS

- G. Anushya *
- T.H. Freeda *

Abstract

Urinary stone is defined as any object resulting from a liquid solid phase transition in urine. Brushite (calcium hydrogen phosphate dihydrate, $CaHPO_4.2H_2O$) and Struvite (ammonium magnesium phosphate hexahydrate, $MgNH_4PO_4.7H_2O$) are important constituents of urinary stone crystals. The brushite and struvite crystals were grown by single diffusion technique in silica gel at room temperature. In order to understand the effect of siddha and allophatic medicines on the formation of brushite and struvite crystals, we have grown these crystals without and with adding these medicines separately with 10mg concentration in each case. The grown crystals were characterized by total product mass determination, X-ray diffraction, UV-Visible and Dielectric studies. Dielectric study showed that the value of dielectric constant is found to decrease as the frequency increased. The dielectric loss and AC conductivity were also evaluated at different frequency.

Keywords: Brushite and Struvite, Gel method, XRD, UV-Vis and Dielectric Studies.

INTRODUCTION

Most of the living beings form crystal. There are many minerals present in the dissolved form in the human body. The body fluids contain minerals at various levels of saturation. When the body fluid gets supersaturated with minerals crystallization takes place. These crystals have both beneficial as well as pathological effects on human body. The major beneficial roles of mineralization are the formation of bones and teeth, which consist of oriented micro crystals of hydroxyapatite. Our sense of balance and acceleration is dependent upon small calcite crystals present in the inner ear. The pathological effect results in the crystal deposition diseases. Crystals deposition disease may be defined as a pathological condition associated with the presence of crystals which then contribute to the tissue damage [1]. Gallstones consist mainly of cholesterol with small quantities of calcium phosphates and calcium carbonates [2]. Clusters of small crystals formed in the urinary system give rise to urinary calculi which consists primarily of calcium oxalates, calcium phosphates, uric acid, urates, etc [3].

Each type of urinary stone has a different cause for its formation. Approximately 80% of urinary stones contains calcium in combination either oxalate or phosphate. Calcium phosphate occurs as hydroxyapatite or brushite. The clusters of small crystals formed in the urinary system give rise to urinary (renal) calculi, which consist primarily of different forms of calcium, such as

* Physics Research Centre, S.T. Hindu College, Nagercoil – 629002, Tamil Nadu, India.
Corresponding author's E-mail : g.anushya7@gmail.com, Phone: 91-4652-225940.

calcium oxalates, calcium phosphates, uric acid, etc. Renal stones occur when there is a decrease in urine volume or an excess of stone forming substances are present in the urine. The formation of stones within the urinary tract in men represents a potential complication of many varied metabolic disorders.

Of the various formed in the human body, deposition of matter in renal system has become a serious threat to human life and affects a large population world-wide. This makes it a social and economic problem of considerable magnitude. The condition of having stones in the kidney is termed as nephrolithiasis.

Urology benefits greatly because concretions of mineral matter (calculi) are common in the urinary system. The value of mineralogical analysis of urinary material was first described by prien and frondel [4]. When the mineral level of the body fluid increases, automatic mineral deposition starts [5]. Kidney stones themselves consist of approximately 98% crystalline material [6-7]. Almost 50 types of stones have been recognized on the basis of the crystalline composition [8]. The most common type of stones in clinical practice are calcium salts, ie calcium oxalate and calcium phosphate followed by uric acid, struvite and cystine [9-10]. Most crystals do not contain one single crystal but rather a mixture of several different types with one or two that are predominant [11].

In the present study, an attempt has been made to understand the effect of siddha (Neem leaf powder and thribalachooranam tablet) and allopathic (Daonil and Glycomet) medicines on the growth of brushite and struvite crystals. Both of these crystals were grown by single diffusion gel growth technique and the growth influencing study of the brushite and struvite crystals in the presence of different medicines were carried out. The grown crystals were characterized by total mass determination, XRD, UV-Vis and Dielectric studies.

EXPERIMENTAL

The single diffusion gel growth method was employed to study the growth and promoting or inhibiting behavior of brushitestruvite crystals using different medicines. Sodium metasilicate (SMS) solutions of specific gravity 1.03g/cc were used to prepare the gel. All the chemicals used for this technique are AR grade. Glass test tubes of 25mm diameter and 140 mm length were used as the crystallization apparatus.

For growing brushite crystals, sodium metasilicate solution of density 1.03 g/cc was prepared and the desired pH (6.5) was obtaining by adding 1.5 Morthophosphoric acid drop by drop. After gelation in about 12 hours and gel aging 24 hrs, the supernatant solution namely Calcium Chloride solution (5ml) of 1.5M and Calcium Acetate (15ml) of 1.5M concentration was gently poured over the set gel column. After pouring supernatant solution, the test tubes were capped with airtight stopples. In the same way, 1.5 M calcium chloride and calcium acetate prepared with 10 mg of siddha (Neem leaf powder) and 10 mg of allopathic (Daonil) medicines were gently poured over the gel medium in test tube without disturbing the latter to study the influence of added medicines on the growth of brushite crystals.

For growing struvite crystals, aqueous solution of Ammonium Dihydrogen Phosphate (ADP) $\{NH_4H_2PO_4.2H_2O\}$ of 1.5M concentration was mixed with the SMS solution in appropriate amount so that the pH value 7 could be set for the mixture. After gelation, 10 ml supernatant solution of pure 1.5 M magnesium acetate $\{C_4H_6MgO_4.4H_2O\}$ was added.

The siddha and allopathic medicines namely thribalachooranam tablet and glycomet of 10 mg were added along with 1.5 M magnesium acetate separately to study the influence added medicines on the growth of struvite crystals.

Brushite crystals of rectangular platelet and struvite crystals of needle shape were obtained. The grown samples were harvested after 3 weeks by decanting the test tubes and the gel was removed and subsequently washed with double distilled water and dried for removing the moisture content. The harvested crystals (including tiny crystals) were weighed to obtain the mass of the products got after crystallization.

The XRD data were collected using an automated X-ray diffractometer with CuKα radiation (λ = 1.54060 Å). The UV-visible absorption spectra of the samples under investigation were recorded using UV-Vis Double Beam Spectrophotometer 2201 in the range 200-600 nm. The capacitance and the dielectric loss of pellets of samples were measured using an Agilent 4284ALCR at different temperatures and at different frequencies ranging from 20 Hz to 1 MHz.

The dielectric constant of the crystals was calculated using the formula,

$$\varepsilon_r = C_c/C_a$$

Where, $C_{c\ is}$ the capacitance of the crystal and C_a is the capacitance of the air medium of the same dimension as the crystal.

The a.c. conductivity wascalculated using the equation,

$$\sigma_{ac} = 2\pi f\varepsilon\tan\delta$$

Where 'f' is the frequency and 'tan δ' is the loss tangent.

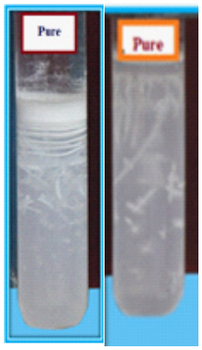

Fig 1: Brushite and struvite crystals grown in gel media

Fig 2: Brushite + 10 mg Daonil

Fig 3: Brushite + 10 mg Neem leaf powder

Fig 4: Struvite + 10 mg Glycomet

Fig 5: Struvite + 10 mg Thribala chooranamtablet

RESULTS AND DISCUSSION

In both the cases, it was observed that the incorporation of siddha medicines namely neem leaf powder (B_1) and thribalachooranam tablet (S_1) and allopathic medicinesnamely, daonil (B_2) and glycomet (S_2) caused an decrease in the number of grown brushite and struvite crystals and their average size.

By carefully observing the shape, size, transparency and approximate number of crystals obtained and also from the knowledge of their total mass (see the table 1), conclusions were derived regarding the promotery/ inhibitory effect of the siddha and allopathic medicines incorporated and illustrated in Fig 6.

Table 1: Total mass of Grown Crystals

Medicine and Dosage	Total Mass of Crystals Formed (g)
a) Pure brushite	1.643
b) 10 mg of Neem leaf powder added brushite (B_1)	0.936
c) 10 mg of Daonil added brushite (B_2)	1.133
d) Pure struvite	0.9592
e) 10 mg of Thribalachooranam added struvite (S_1)	0.703
f) 10 mg of glycomet added struvite (S_2)	0.776

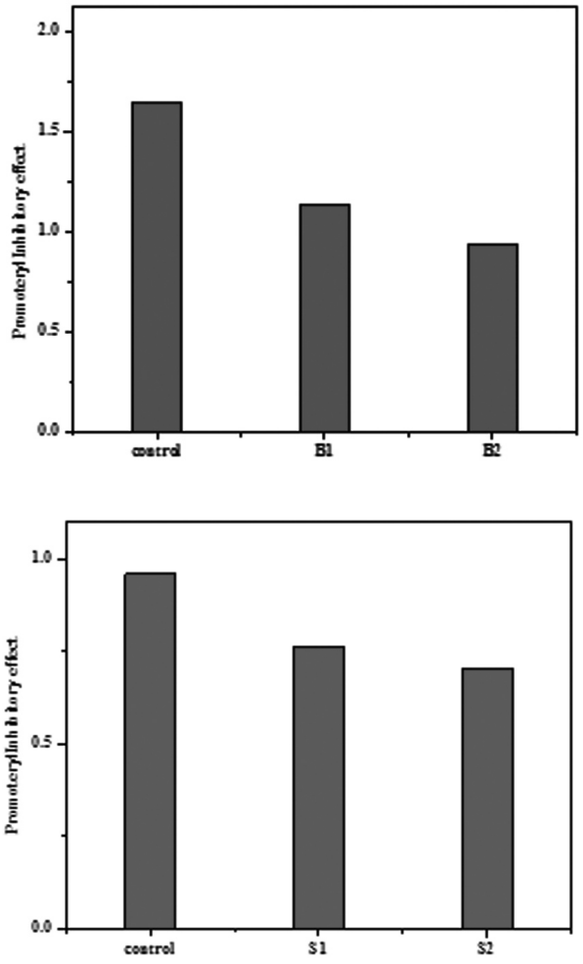

Fig 6 : Promotery/Inhibitory Effect of Brushite and Struvite Crystals

X-Ray Diffraction Analysis

The XRD results revealed the crystalline property of crystal. The XRD patterns of all the samples are shown in the figures (7-12) and the values of h, k, l in brackets are provided. Using the observed 2θ (Bragg angle) and d (inter planar spacing) all the reflections were indexed. The data were indexed following the procedures of Lipson and Steeple [12]. The unit cell parameters and volume (V) obtained in the present study are provided in the Table 2.

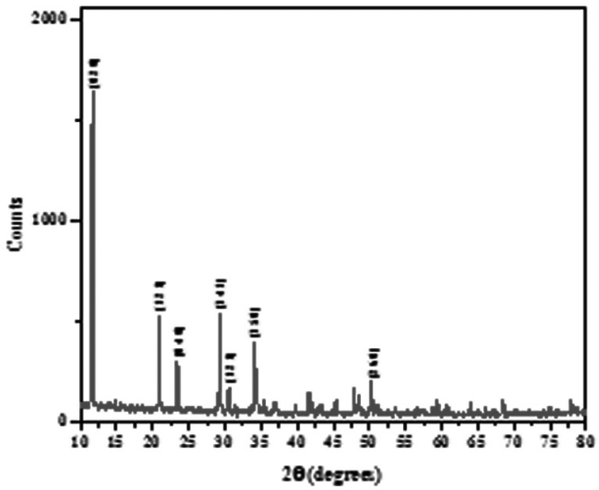

Figure 7: XRD Pattern of Pure Brushite Crystal

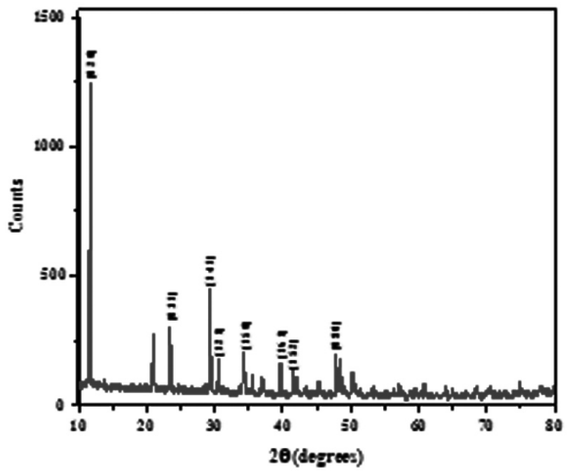

Figure 8: XRD Pattern of 10 mg of B_1 medicine Addedbrushite Crystal

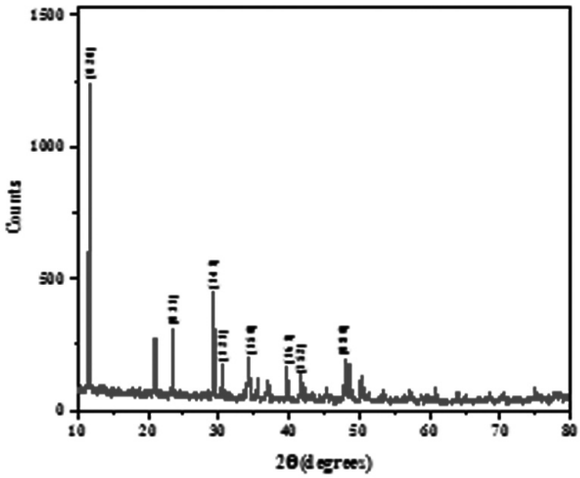

Figure 9: XRD Pattern of 10 mg of B_2 medicine

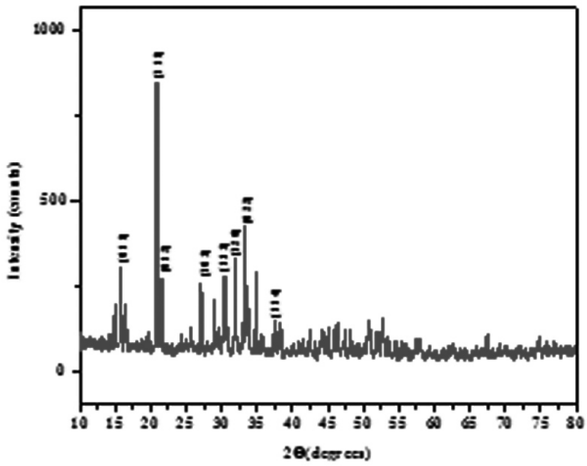

Figure 10: XRD pattern of pure added brushite crystal struvite crystal

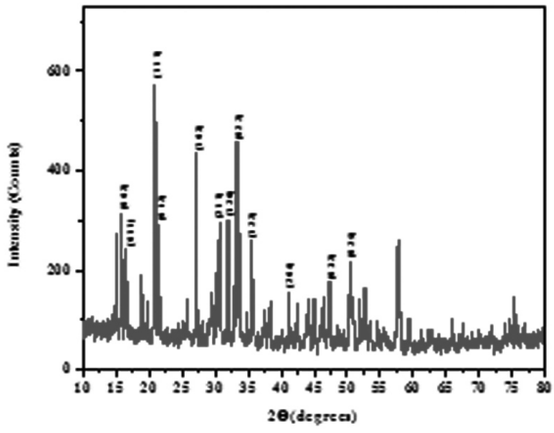

Figure 11: XRD pattern of 10 mg of S_1 medicine added struvite crystal

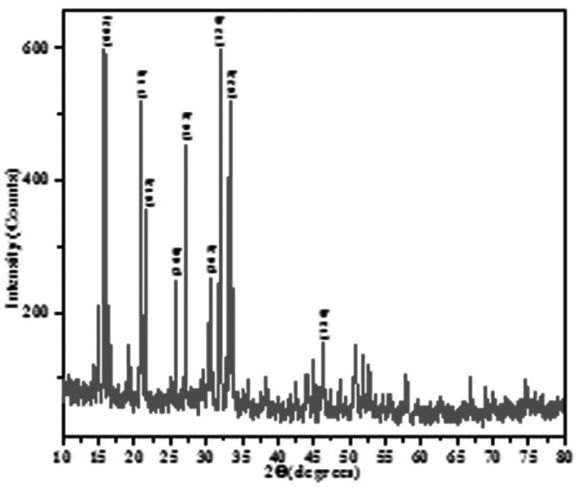

Figure 12: XRD Pattern of 10 mg of S_2 Medicine Added Struvite Crystal

Table 2: Unit Cell Parameters and Volume of Pure and Various Medicines Added Brushite and Struvite crystals

Samples	Unit Cell Parameters			Unit Cell volumeV
	A (Å)	b (Å)	c (Å)	$(Å)^3$
Pure Brushite	5.2125	15.1781	5.605	443.444
10 mg of B_1	5.1825	15.253	5.5538	439.020
10 mg of B_2	5.2272	15.1265	3.8909	307.650
Pure Struvite	6.6950	6.1500	11.2058	461.390
10 mg of S_1	6.7073	6.1927	11.2451	467.079
10 mg of S_2	6.9818	6.1538	11.2060	481.461

From the XRD analysis it is found that the grown brushite crystals crystallizes in the monoclinic structure and the CHPD crystals are known to crystallize in the orthorhombic system. The lattice parameters are in good agreement with the literature values for brushite [JCPDS no. 72-0713] and struvite (JCPDS No.77-2303). The intensity of the diffraction peaks indicates that the samples were well crystallized. The result indicates that for the medicine added CHPD samples the crystal structure remains unaltered with no significant lattice distortion. The diffraction peaks of the MAPH crystals grown with various medicines were strong and sharp when compared to that of pure system. Due to incorporation of medicines in CHPD and MAPH crystals, change in the values of unit cell parameters and cell volume are noticed and this changes are due to the incorporation of the medicines in the lattice of these crystals.

UV-Visible Absorption Analysis

UV-Vis spectrum occurs due to the electronic transistions of the molecule. Figure shows the plots of absorbance vs wavelength (nm) for pure and medicine substituted brushite and struvite samples.

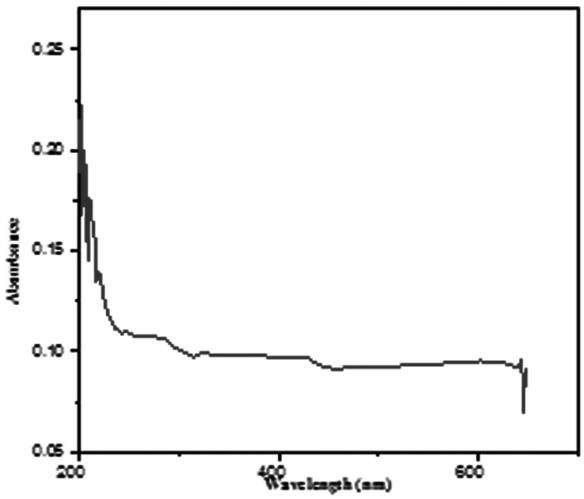

Figure 13: UV-Vis Spectra of Pure Brushite

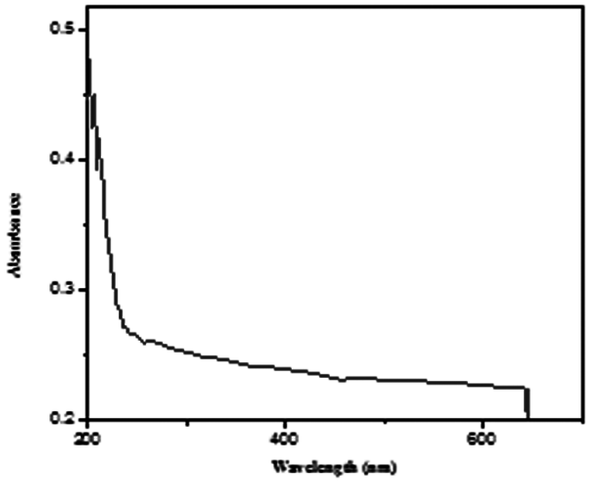

Figure 14: UV-Vis spectra of B_1 Medicine Crystaladded Brushite Crystal

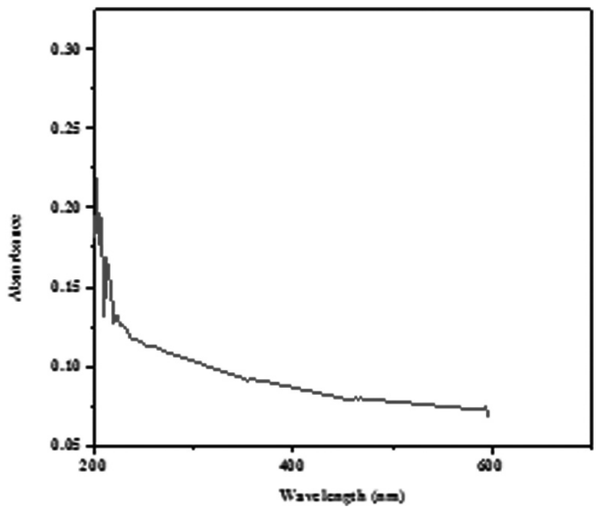

Figure 15: UV-Vis spectra of B_2 Medicine Added Brushite Crystal

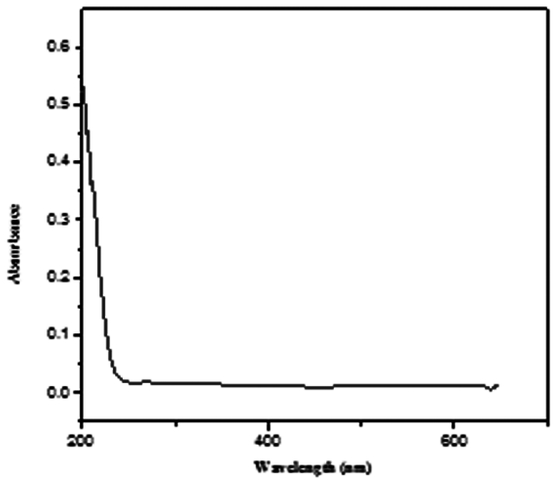

Figure 16: UV-Vis spectra of pure struvite crystal

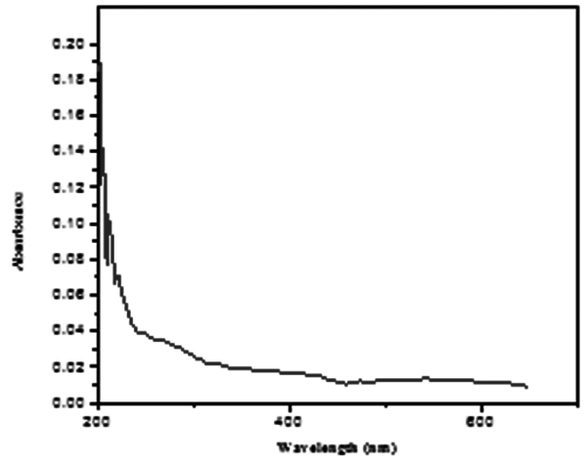

Figure 17: UV-Vis spectra of S_1 Medicine Added Struvite Crystal

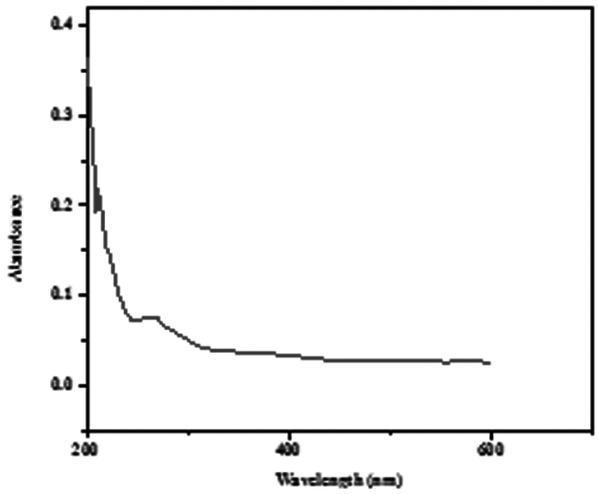

Figure 18: UV-Vis spectra of S_2 Medicine Added Struvite Crystal

The UV-Visible spectrum gives information about the structure of the molecule that the absorption of ultraviolet and visible light involves promotion of electrons in σ and π orbital from the ground state to higher energy state. It was found that there is no significant absorption in the entire spectrum of study for both the pure and medicines added struvite, which means that the grown crystals were transparent in the entire spectral range between 370 and 600 nm respectively. The band gap for the brushite and struvite crystals grown in the control system was found to be 9.16 eV and 5.9eV. For the medicines added brushite and struvite crystals, the band gap energy decreases.

Dielectric Studies

The investigation of the dielectric property is an elegant experimental tool to probe the electrical properties of a crystalline solid. The study of dielectric characteristics indicates the response of the grown material to an applied electric field. The electrical parameters, vizε_r, tanδ and σ_{ac} observed in the present study are shown in Figs (19-36).

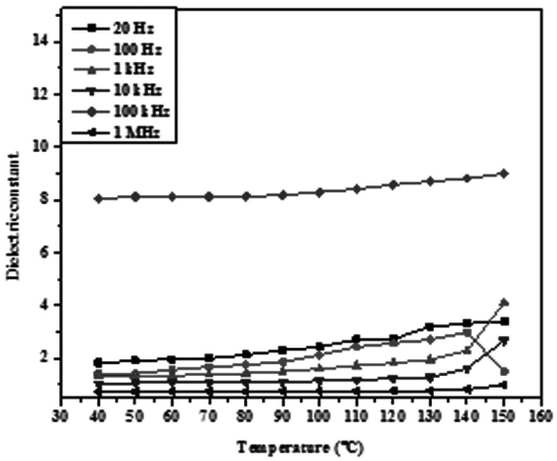

Figure19: Dielectric Constant of Pure Brushite Crystals

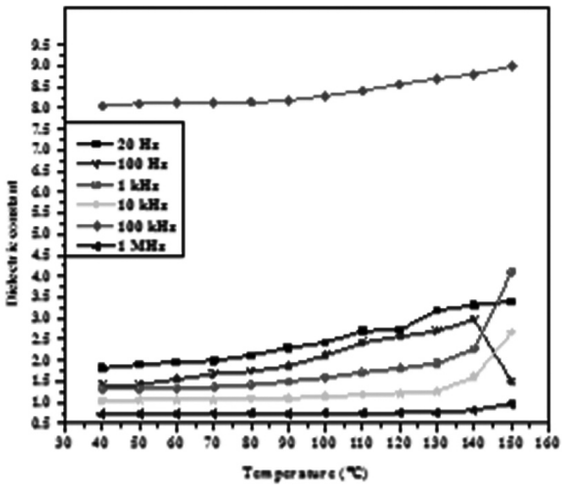

Figure 20: Dielectric Constant of B_1 medicine Added Brushite Crystals

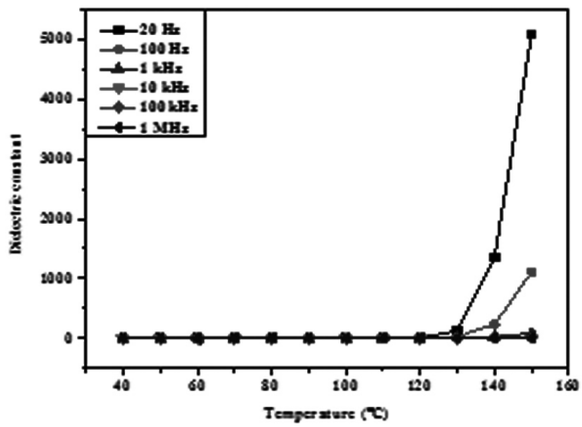

Figure 21: Dielectric Constant of B_2 Medicine Added Brushite Crystals

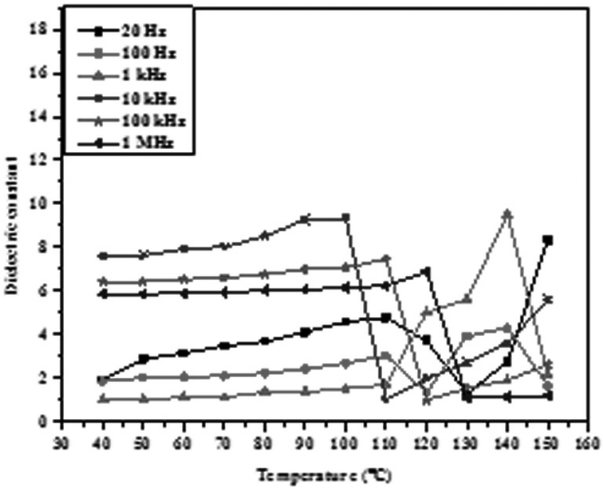

Figure 22: Dielectric Constant of Pure Struvite Crystals

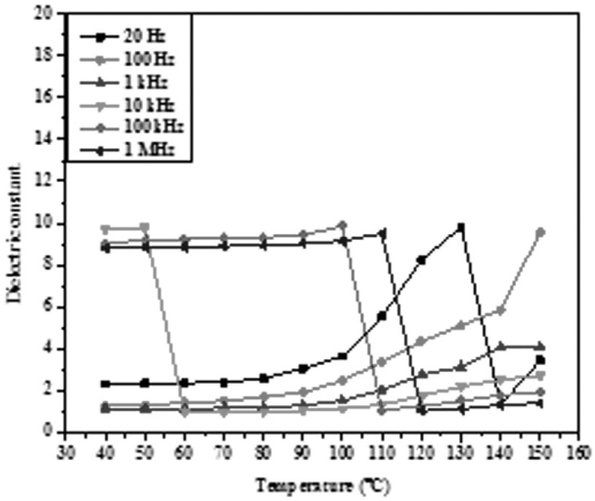

Figure 23: Dielectric Constant of S_1 Medicine Added Struvite Crystals

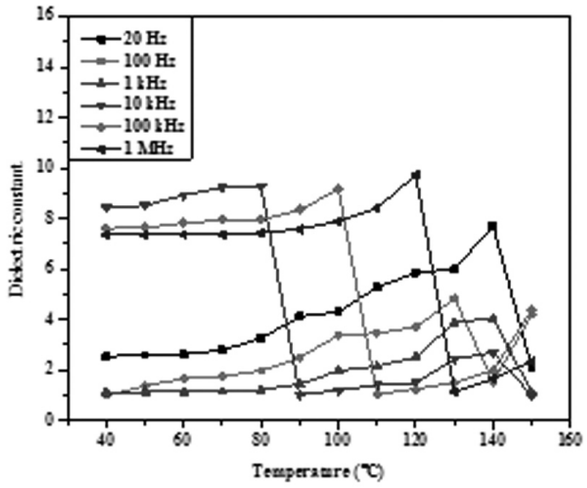

Figure 24: Dielectric Constant of S_2 Medicine Added Struvite Crystals

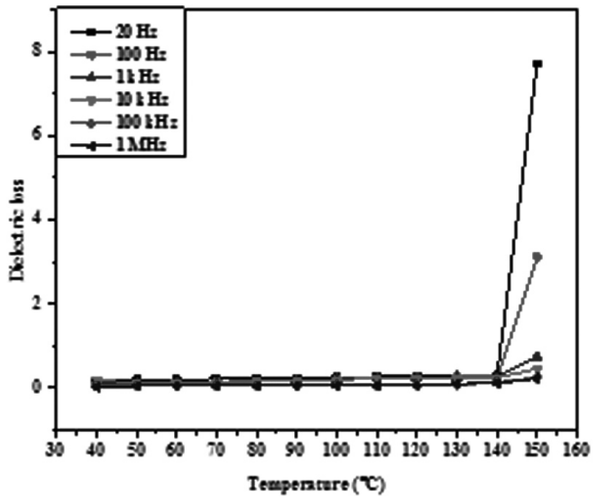

Figure 25: Dielectric Loss of Pure Brushite Crystals

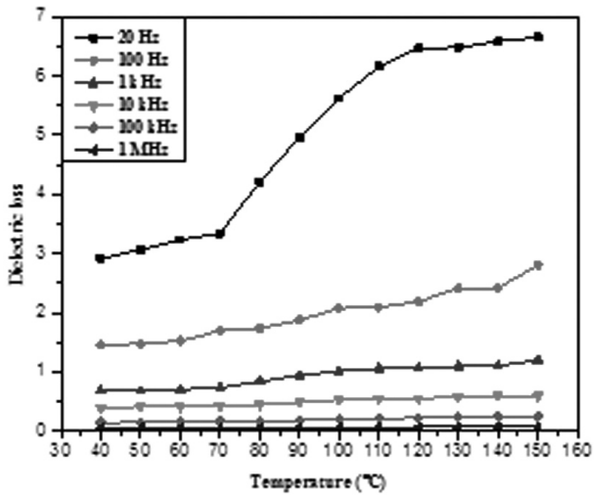

Figure 26: Dielectric Loss of B_1 medicine Added Brushite Crystals

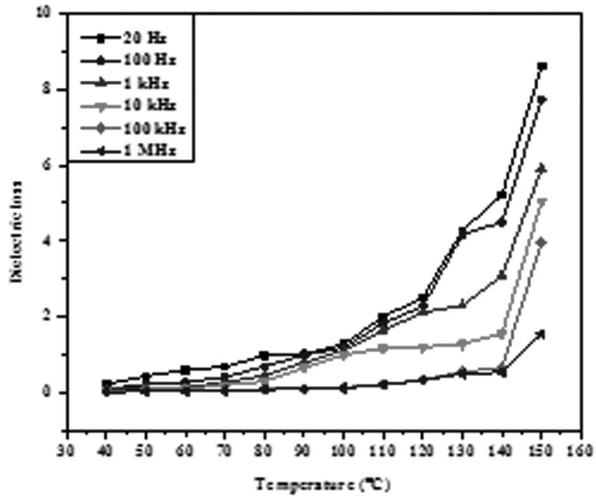

Figure 27: Dielectric Loss of B_2 Medicine Added Brushite Crystals

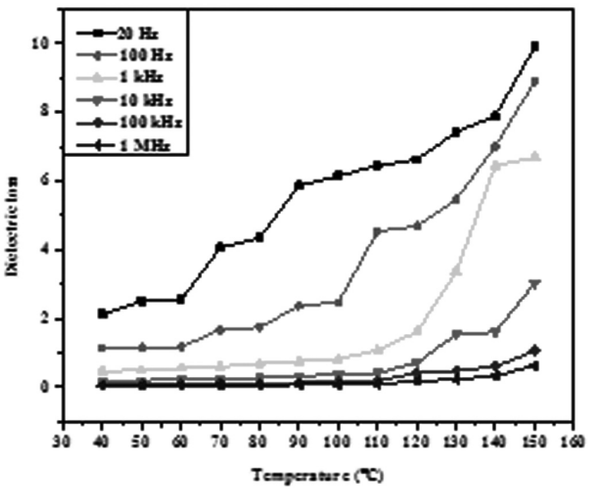

Figure 28: Dielectric Loss of Pure Struvite Crystals

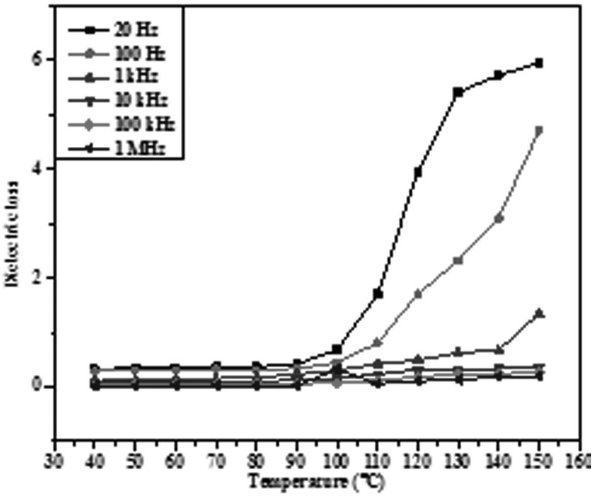

Figure 29: Dielectric Loss of S_1 Medicine Added Struvite Crystals

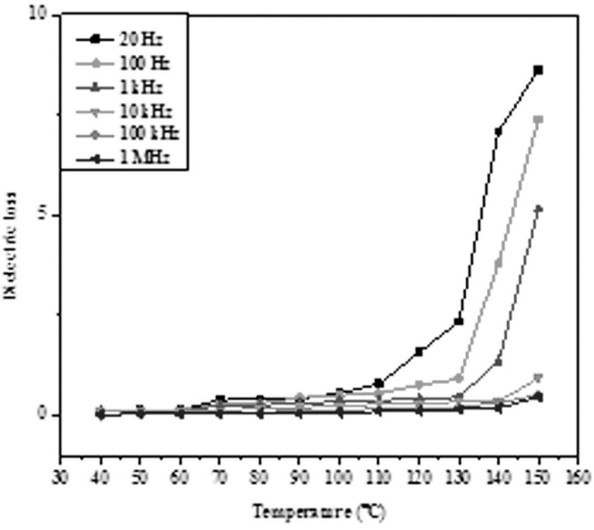

Figure 30: Dielectric Loss of S_2 Medicine Added Struvite Crystals

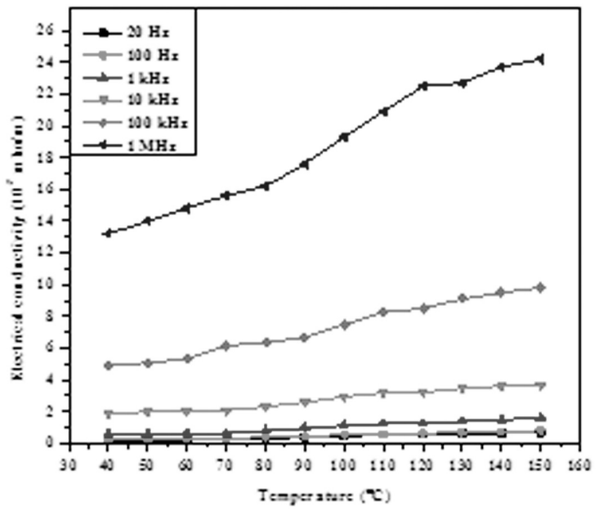

Figure 31: AC Electrical Conductivity of Purebrushite Crystals

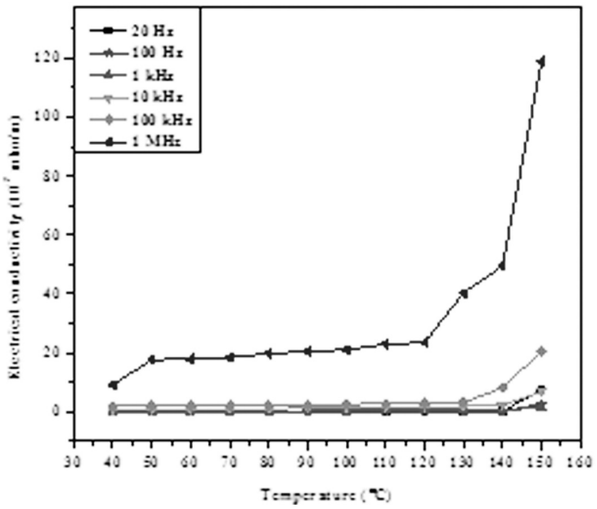

Figure 32: AC Electrical Conductivity of B_1 Medicine Added Brushite Crystals

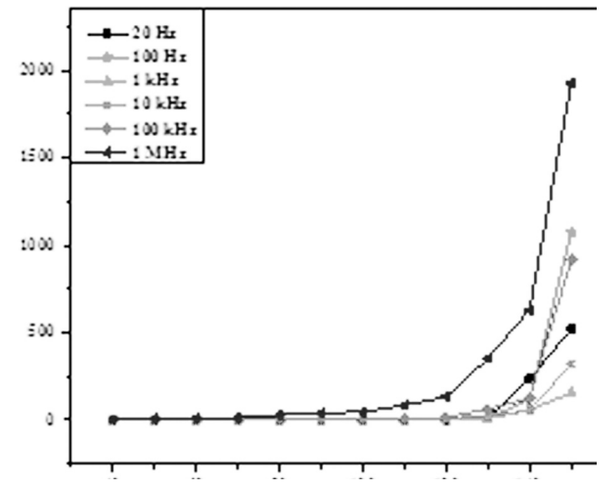

Figure 33: AC Electrical Conductivity of B_2 Medicine Added Brushite Crystals

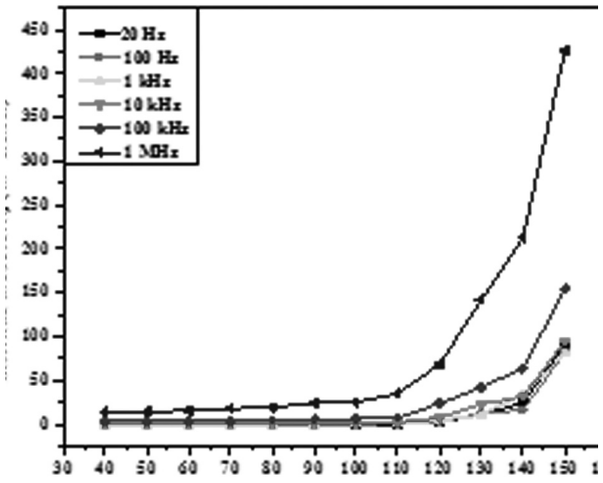

Figure 34: AC Electrical Conductivity of Pure Struvite Crystals

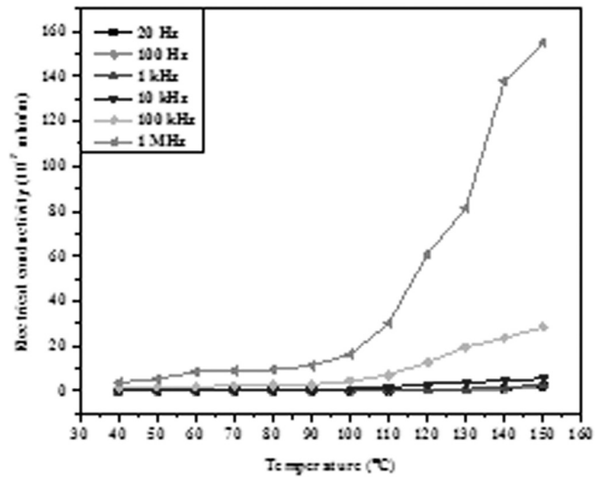

Figure 35: AC Electrical Conductivity of S_1 Medicine Added Struvite Crystals

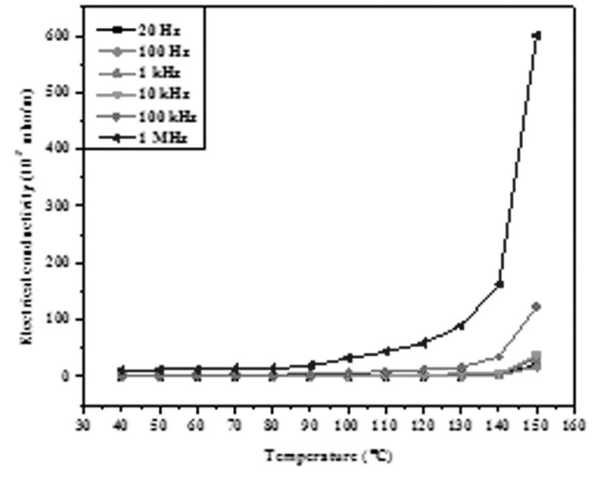

Figure 36: AC Electrical Conductivity of S_2 Medicine Added Struvite Crystals

From the figures it was noticed that the dielectric constant increases with temperature and decreased with the increasing value of frequency of applied field. This may be due to dipoles do not comply with the rapidly varying electric field.

The decrease in dielectric constant with increase in frequency is a normal dielectric behaviour and can be explained on the basis of polarization mechanism. It is a known fact that polarization arises from induced electric dipoles. There are four basic mechanism of polarization which in order of their response time include, the electronic polarization (which is always present), ionic polarization (which is responsible for differences in dielectric constant of materials), it includes ion jump (which is distinguished from ionic polarization because of its totally different response time), orientation polarization (also called dipolar polarization) and space charge or diffusional polarization. The electronic and ionic polarizations do not decrease significantly with the rise in temperature and hence are temperature independent [13]. However, the space charge and dipolar polarization are relaxation towards polarization was so insignificant at lower temperatures and higher frequencies that one may take it as negligible but it was significant in the lower frequency region, as the temperature increases the contribution from space charge effect towards polarization may have a tendency to increase.

The dielectric loss was found to be dependent on the frequency of applied field at room temperature. It was noticed that the dielectric loss were found to be decreased with the increasing value of frequency of applied field.

It is noticed that a.c conductivity increases as the frequency and temperature increases. The electrical conduction is expected to occur due to hopping of protons through hydrogen bondings through hydrogen vacancies.

CONCLUSION

The brushite and struvite crystals without and with medicines were successfully grown by single diffusion gel growth technique. The XRD studies confirmed the structural identity of the grown brushite and struvite crystals. The UV-Vis analysis showed that the energy gap decreases for the medicines added brushite and struvite crystals. The variation of dielectric constant with frequency suggests the decrease in the value of dielectric constant as frequency decreases. It was also found that the doping changes the dielectric behavior of all the crystals. The a.c. conductivity is expected due to the hopping of protons through vacancies in hydrogen bondings. The results obtained in the present study showed that both the siddha and allopathic medicines, have inhibitory effects on the growth of brushite and struvite crystals indicating the double usage of these medicines: one in reducing the sugar and the other in preventing the urinary stone formation. But while comparing allopathic (Daonil and Glycomet) and siddha medicines (Neem leaf powder and Thribalachooranam tablet), one can easily conclude that the siddha medicines give comparatively good inhibition than the others.

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