Comparative Study of Pure and Ocimum Tenuiflorum Added Brushite Crystals

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Abstract**--** Urinary stone disease has become one of the common causes of morbidity and affects upto 5% of the population. Brushite or Calcium Hydrogen Phosphate Dihydrate (CHPD) also known as urinary crystal is a stable form of calcium phosphate. In the present study, the brushite crystals are grown by single diffusion technique in silica gel at room temperature. The effect of herbal extract of Ocimum Tenuiflorum is used as additive to inhibit the nucleation and growth of the brushite crystals. The crystals harvested are characterized by powder X-Ray Diffraction, UV-Visible spectral analysis and Dielectric studies. From the PXRD it is found that the crystallite size is reduced for Ocimum Tenuiflorum added CHPD crystal while comparing with pure CHPD. UV-Vis spectra shows that the optical band gap energy decreases for Ocimum Tenuiflorum added brushite crystals. The frequency and temperature dependence of the dielectric parameters were different for pure and Ocimum Tenuiflorum added crystals and throws light on the polarization mechanisms in these crystals.

Keywords**--** Brushite, Gel Growth, Ocimum Tenuiflorum, W-H Plot, Dislocation density.

I. INTRODUCTION

Nephrolithiasis is a common health problem in worldwide affecting between 2-5% of the population during lifetime at least once. Recent evidence has shown that kidney stones are becoming more common [1]. Many risk factors have been related to this painful and costly disease which includes geographical and weather conditions, race and ethnicity, dietary issues and more recently, the metabolic syndrome are some of the explanations for this rise in the lifetime risk of kidney stones [2-6]. Calcium Hydrogen Phosphate Dihydrate (CHPD, Brushite, and CaHPO4.2H2O) plays a significant role in the formation of urinary calculi. Components forming Calcium Phosphate stone comprise about 15% of the stone forming population and the incidence of calcium phosphate stones may be increasing [7]. The formation of calcium phosphate stones is associated with conditions such as hyperparathyroidism and renal tubular acidosis [8-9]. A considerable percentage of patients, however, experiences recurrent kidney stones with pain, urinary tract infections, and possibly leading to loss of functional renal parenchyma which may ultimately cause even renal insufficiency [10]. Patients suffering from brushite stone disease are less likely to be rendered stone free after surgical intervention; hence, a drug for the prevention of this disease or its recurrence would be of great interest.

In ayurvedic and siddha system of medicine, Ocimum Tenuiflorum (Tulsi) is used to strengthen the function of kidneys. Intake of Tulsi juice and honey regularly for six months will expel the renal stone from the urinary tract [11]. The aim of the present work is to study the influence of Ocimum Tenuiflorum on the crystallization of CHPD under in vitro conditions by single diffusion gel method.

Gel acts as an inert medium during the growth of many crystalline compounds and it is considered as a simplified model to study of the crystallization of biomolecules in-vitro [12]. Moreover, their viscous nature provides simulation of biological fluids in which biomolecules grow.

II. EXPERIMENTAL

In the present work, glass test tubes of 25 mm diameter and 140 mm length are used as a crystallization apparatus. The AR grade chemicals (Merck) are used to grow the crystals. Sodium meta silicate gel is used as the medium to grow brushite crystals at room temperature [13].

1 ml of Herbal extracts of Ocimum Tenuiflorum is added along with the calcium chloride and calcium acetate solutions and the crystals are grown. The crystal takes about 28 days to complete its growth and it appears to be elongated and platy. The grown crystals are collected in the Petridish and the gel is removed by washing with distilled water and then dried by placing it in a filter paper at room temperature. The fine powder of the dried crystals is used for further characterization.

III. CHARACTERIZATION

The powder X-ray Diffraction data are collected using an automated X-ray diffractometer with CuK_α radiation (λ = 1.54060Å). UV-Visible Double Beam Spectrophotometer 2201 is used to get the absorption spectra in the wavelength range 200-600 nm. The capacitance (C_c) and the dielectric loss factor (tan δ) of the pellets of all the grown samples are measured by the conventional parallel plate capacitor method using an Agilent 4284A LCR meter.

IV. RESULTS AND DISCUSSION

Brushite crystals are grown by single diffusion gel growth method. A dense white precipitate has been formed at the gel solution interface within 15 minutes of adding supernatant solution. Liesegang rings have appeared just below the interface within 7 hours. The number of Liesegang rings gradually increased with time and totally 7 rings are observed after 6 days. The transparent platy shape crystals have grown in between the Liesegang rings. It is observed that the addition of Ocimum Tenuiflorum reduces the size and total number of brushite crystals. Figure 1 and 2 shows the grown crystals with and without Ocimum Tenuiflorum in gel media.

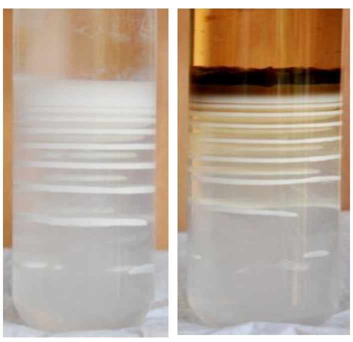


Fig 1: Pure Brushite   Fig 2: Brushite + 1 ml of Ocimum Tenuiflorum

The total mass of the grown crystals is tabulated in Table 1

Table 1: Total mass of grown crystals

Medicinal plant and dosage	Total mass of crystals formed (g)
a) Pure brushite	1.643
b) 1ml of Ocimum Tenuiflorum added brushite (A_1)	0.381

The promoting/inhibitory effect of the Ocimum Tenuiflorum incorporated on the gel medium is shown in figure 3.

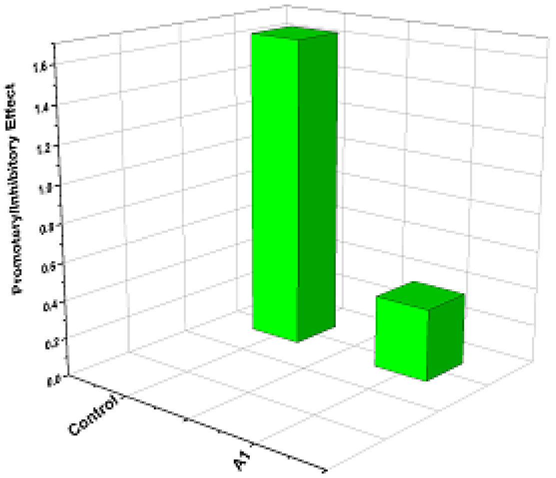


Figure 3: Promotery/Inhibitory effect of Brushite crystals

A. Powder X-Ray Diffraction

Figure 4 shows the powder X-ray diffraction patterns of undoped Brushite crystals and Ocimum Tenuiflorum added brushite crystals respectively. The X-ray diffraction peaks at an angle 2θ are indexed by miller indices corresponding to CHPD (JCPDS no. 72-0713) indicating that the CHPD crystallizes in monoclinic crystal system. The sharp diffraction peaks indicate that the crystallinity of the grown crystals is good. The PXRD patterns reveal the absence of additional phases due to Ocimum Tenuiflorum addition, thus retaining the monoclinic structure. A slight shift in the peak positions indicates the chances of incorporation of additional ions into the CHPD crystal lattice.

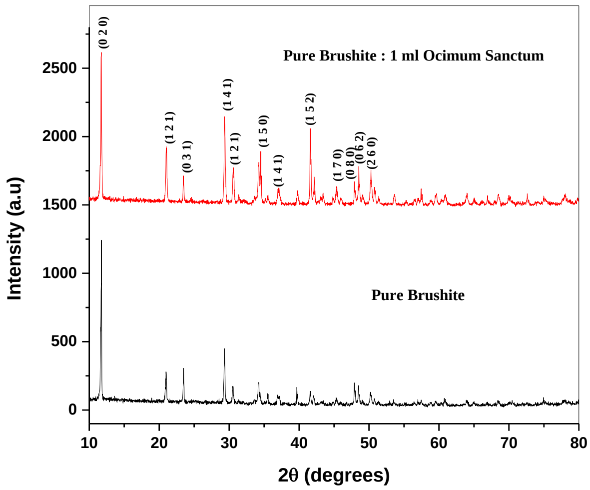


Figure 4: PXRD pattern of pure and Ocimum Tenuiflorum added Brushite crystals

Table 2: Unit cell parameters and volume of pure and Ocimum Tenuiflorum added brushite crystals

Samples	Unit cell parameters			Unit cell volume V (Å)^3
	a (Å)	b (Å)	c (Å)	
Pure Brushite	5.812	15.18	6.239	492.91
1 ml of A_1	5.2037	15.1802	6.2022	489.9316

The average crystallite size of the Brushite crystals is calculated using Scherrer equation,

$$D = \frac{K\lambda}{\beta\cos\theta}$$

Where D is the crystallite size, K (=0.89) is the constant, λ is the wavelength of CuKα radiation, β is the full width at half maximum value and θ is the Bragg diffraction angle. It is found that the average crystallite size increases for Ocimum Tenuiflorum added Brushite crystals (Table 2). The Smaller crystallite size forms higher ultimate stones volume, whereas large crystallites form smaller burden stones [14].

B. Williamson Hall Plot Method

The strain produced by crystal defects are analyzed by using modified Williamson-Hall equation,

$$\beta\cos\theta = \frac{K\lambda}{D} + 4\varepsilon\sin\theta$$

Where λ is the wavelength of X-ray radiation, β is the full width at half maximum, θ is the Bragg diffraction angle, where D is the effective crystallite size, ε is the effective value of micro strain. A plot is drawn with 4sinθ along the X-axis and βcosθ along the Y-axis. The particle size and strain are calculated using linear fit. The crystallite size was estimated from the Y-intercept and the strain (ε) from the slope of the linear fit to the data [15].

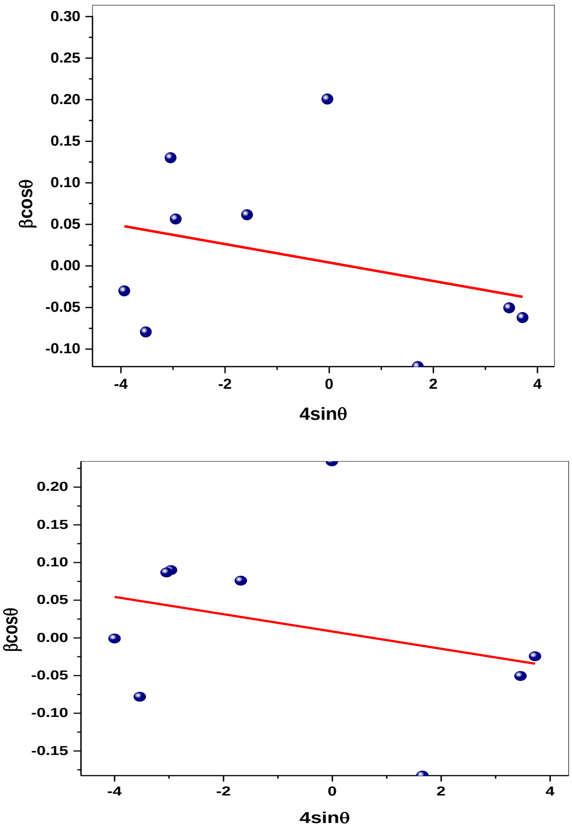


Figure 5: Williamson Plot for (a) Pure Brushite crystals (b) Ocimum Tenuiflorum added Brushite crystals

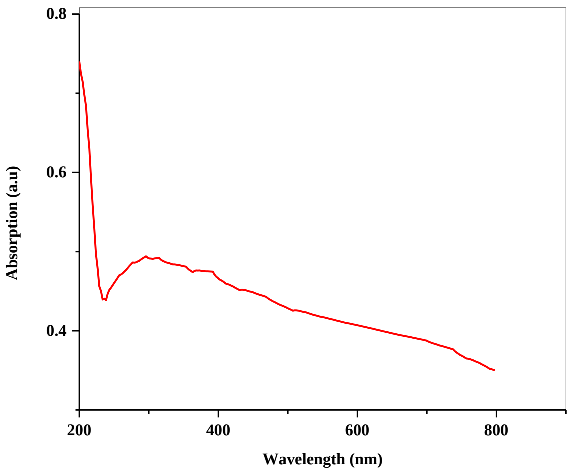
Table 3: Structural parameters of pure and Ocimum Tenuiflorum added Brushite crystals

Sample	Crystallite size (nm) (Scherrer formula)	Crystallite size (nm) (W-H plot)	The dislocation density($x10^{14}$) (lines/m^2)	Strain ε ($x10^{-3}$)
Pure Brushite	53	41	5.948	11.1
A_1	375	496	0.041	1.147

From the above table it is observed that the strain value decreased with increasing crystallite size for Ocimum Tenuiflorum added brushite crystals. Also due to the addition of Ocimum Tenuiflorum the diffraction peaks shifted towards higher angle which may be responsible for the decrease in the value of lattice constants.

UV- Vis Spectral Analysis

The UV-Vis absorption spectra for pure and Ocimum Tenuiflorum added brushite crystals are shown in figure 6 (a & b).



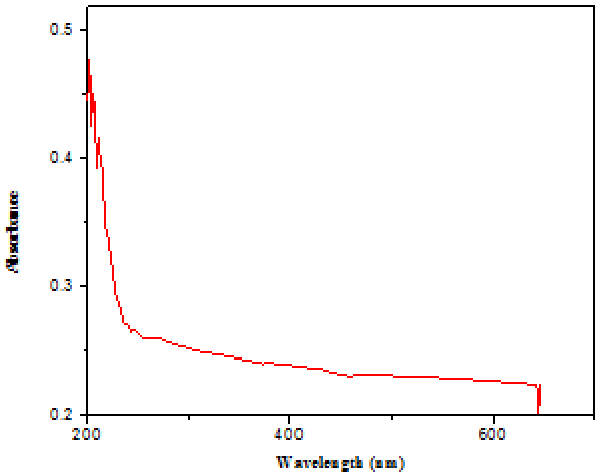


Figure 6: UV-Vis absorption spectra for (a) pure CHPD (b) Ocimum Tenuiflorum added CHPD

In ultraviolet and visible spectroscopy, the transitions which result in the absorption of electromagnetic radiation in this region of the spectrum are transitions between electronic energy levels. The absorbance was reduced drastically between the wavelength of 210 nm and 800 nm indicates well transparency of crystalline nature. Further, from the spectra of Ocimum Tenuiflorum substituted samples the characteristic absorption band occurs at 231 nm. The band gap for the pure brushite crystal is found to be 9.16 eV. For the medicines added brushite crystals, the band gap energy decreases to 5.39 eV.

Dielectric studies

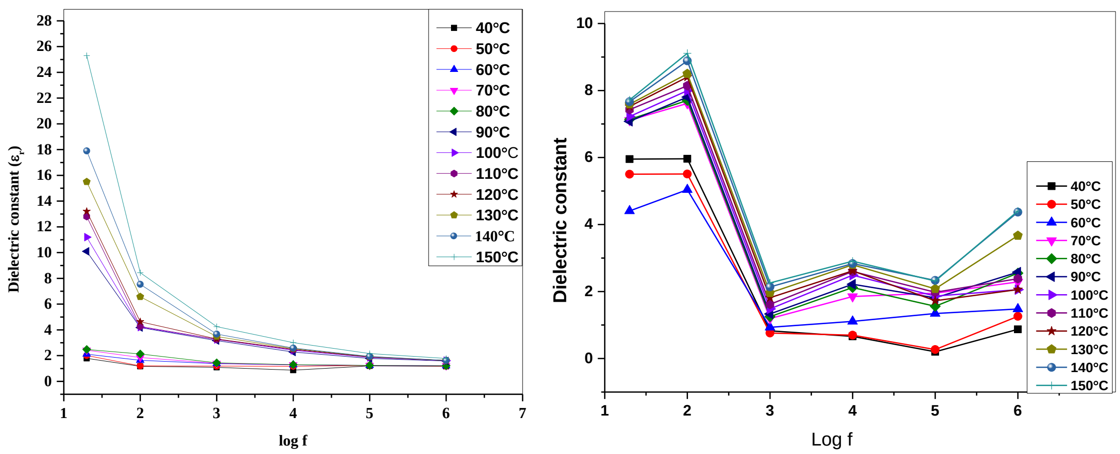
The dielectric constants was calculated using the relation

$$\varepsilon_r = C_c/C_a.$$

The AC electrical conductivity (σac) was calculated using the formula:

$$\sigma_{ac} = \varepsilon_0\varepsilon_r\omega\tan\delta.$$

Where ε_0 is the permittivity of free space (8.85 x 10^{-12} $C^2N^{-1}m^{-2}$) and ω is the angular frequency (ω = 2πf, where f is the frequency).



(a) (b)

Fig 7: Dielectric constant for (a) Pure CHPD (b) Ocimum Tenuiflorum added CHPD

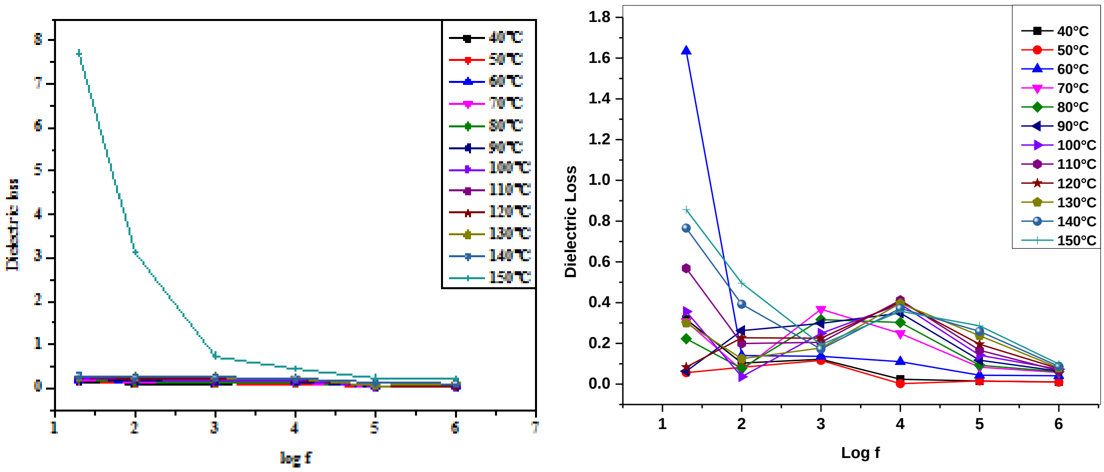


Figure 8: Dielectric loss for (a) Pure CHPD (b) Ocimum Tenuiflorum added CHPD

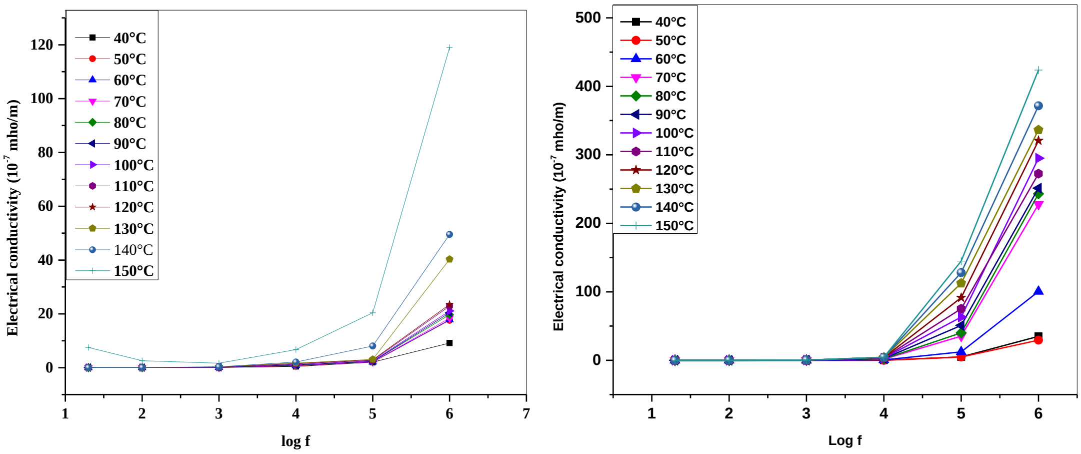


Figure 9: AC Electrical conductivity for (a) Pure CHPD (b) Ocimum Tenuiflorum added CHPD

Figure 7 (a &b) shows dielectric constant as a function of frequency for different temperatures ranging from 40ºC to 150ºC in case of CHPD and Ocimum Tenuiflorum added CHPD crystals. The decrease in dielectric constant with increase in frequency is a normal dielectric behaviour and can be explained on the basis of polarization mechanism. There are four polarization mechanisms which contribute to the net polarization in a given material i.e., ionic polarization, electronic polarization, orientational polarization and space charge polarization. The ionic and electronic polarization decreases with increase in temperature e.g., the increase in temperature increases the ionic distances which affect both the ionic as well as electronic contribution to the polarization both becoming weaker as the temperature rises [16]. From figure it is observed that at low frequency and at higher temperature (say 150ºC) the value of dielectric constant is higher and this may be due to the occurrence of space charge polarization. The high value of dielectric constant at low frequencies may be due to the presence of all the four types of polarizations and the low value of dielectric constant at high frequencies may be due to the loss of significance of some of these polarizations gradually. The variation of dielectric loss (tanδ) with frequency for both samples is shown in figure 8 (a & b) respectively. The low dielectric loss observed at higher frequencies indicates the low power dissipation in the crystals.

The frequency dependent ac conductivity curve is plotted incase of CHPD and Ocimum Tenuiflorum crystals are shown in figure 9(a & b). At low frequencies, the bulk a.c conductivity (σ_0) is almost frequency independent but at higher frequencies, the a.c conductivity increases following power law behaviour such that $\sigma_{ac} = A\omega^s$ [17].

CONCLUSION

The inhibitory effect of Ocimum Tenuiflorum on the growth of Brushite crystals is evaluated using single diffusion gel growth technique. The present study reveals that the herbal extract of Ocimum Tenuiflorum is very effective to inhibit the growth of brushite crystals in vitro gel conditions. From the PXRD it is found that the crystallite size is increased for Ocimum Tenuiflorum added brushite when comparing with the pure CHPD. Also it confirms the incorporation of additional ions in the Ocimum Tenuiflorum leaf extracts into the host of CHPD. A UV-Vis spectrum shows that the grown crystals are transparent in the entire UV-Vis region. The decrease in dielectric constant with increasing in frequency is due to the presence of space charge polarization. The ac electrical conductivity increases with increase in frequency.

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