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EFFECT OF GREEN TEA ON THE GROWTH OF BRUSHITE CRYSTALS

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Abstract-Urolithiasis is a global problem affecting mankind for several centuries. It is also called Nephrolithiasis or kidney stones; Urolithiasis is the presence of uroliths/calculi (stones) in the urinary tract. Urinary stones have been found to contain phosphate, uric acid, magnesium ammonium phosphate with apatite and struvites. Among the urinary stones, calcium containing stones are the common comprising about 75% of all urinary calculi, which may be in the form of pure calcium oxalate or calcium phosphate and a mixture of both. Brushite [CaHPO4· 2H2O] or calcium hydrogen phosphate dihydrate (CHPD) also known as urinary crystal is a stable formof calcium phosphate. The brushite crystals were grown by the single diffusion gel growth technique.The effect of green tea on the crystallization of brushite in sodium metasilicate gel has been studied at room temperature.The crystals were characterized by various experimental techniques, viz. powder XRD, UV-Vis, Dielectric studies and total mass determination. Dielectric study shows that the value of dielectric constant is found to be decreased as the frequency increased.
Keywords: Brushite, Gel method, X-ray diffraction, UV-Vis spectrum, Dielectric studies.

1. Introduction

Urinary calculi are the hard masses developed from crystals that separate from the urine and build up on the inner surfaces of the kidney or urinary tract [1]. Many factors affect the urinary calculi formation; particularly, different mineral metabolisms are the most important factors in the formation of urinary calculi [2]. Stones are composed mainly of a crystalline component. The formation of crystalline particle in tubular fluid as well as in urine comprises two major physico chemical aspects: a thermodynamic one including supersaturation, which results in nucleation of microcrystals, and a kinetic one comprising rate of crystal nucleation, growth, aggregation and phase transformation, which in turn depends on solution supersaturation. The rates of the four kinetic processes will determine phase, shape, size and number of crystals formed [3]. Calcium oxalates, phosphates, and their hydrates are very common in calcium renal stones. Some of the oxalates arefound in either pure or in mixed form with phosphate and also reported with uric acid or ammonium urates [4, 5]. The brushite mineral is found under various pathological conditions including kidney stones, some forms of arthritis, and caries [6, 7]. It was reported that the mineral deposits in the kidney contain various form of calcium salts such as calcium oxalate and calcium phosphate [8]. The calcium phosphate minerals are thought to be the initiator of stone formation in the kidney and urinary bladder, under the favorable physiological environment.

The ultimate control of urolithiasis requires a proper application of both the approaches of stone removal and drug therapy. Surgical therapy just removes the stone already present. Drug therapy is mainly aimed at the inhibition of growth of existing stone and the formation of new stones. The use of drugs prevents stone recurrence, avoids renal colic, reduces the need for surgery and may correct the extra renal manifestations of systemic diseases. The cost of drug is considered to be negligible when compared to the cost of stone removal [9]. Hence, it is important to understand the mechanism of stone formation and identification of the inhibitors and promoters of different crystalline materials present in the urinary calculi.

Gel acts as an inert medium during the growth of many crystalline compounds and it acts as an ideal medium in the study of the crystallization of biomolecules in-vitro [10]. Moreover, their viscous nature provides simulation of biological fluids in which biomolecules grow.

In this context, the present study is undertaken to assess the effect of the green tea on the growth inhibition of brushite or calcium hydrogen phosphate dihydrate($CaHPO_4.2H_2O$, CHPD) using single diffusion gel growth technique.

2. Experimental

The single diffusion gel growth method is employed to study the growth and promoting or inhibiting behavior of brushite crystals using green tea.This technique consists of incorporating one reagent in the gelling mixture and later allowing another reagent to diffuse into the gel, leading to high supersaturation to initiate nucleation and crystal growth [11]. In this method, glass test tubesof size 140 mm length and 25 mm in diameter are used for the crystallization.Sodium meta-silicate solution of specific gravity1.03 was acidified by adding appropriate amount of orthophosphoric acid in which the pH value is maintained as 6.5.Once when the solution undergoes a gelation phase, the supernatant solution ofcalcium chloride and calcium acetate of 1.5 Mconcentration is carefully poured on the set gels. After pouring supernatant solution, the test tubes were capped with airtight stopples.

The growth of the crystals are observed in an hourly basis.After 48 hours the growth is seen prominent. In addition to that, another sample of 1.5 M calcium chloride and calcium acetate with 0.5 ml of green tea is prepared with the gel medium without disturbing the system. The grown crystals are of elongated, platy like and star shaped. The grown

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crystals are carefully harvested and rinsed in distilled water and then dried in the atmosphere on a filter paper. These grown crystals are characterized by powder XRD, UV-Vis analysis. The XRD data were collected using an automated X-ray diffractometer with CuK_α radiation (λ = 1.54060 Å). The UV-visible absorption spectra of the grown samples were recorded using UV-Vis Double Beam Spectrophotometer 2201 in the range 200-600 nm.
Dielectric studies are also carried out for the crystals. The capacitance and the dielectric loss of pellets of samples are measured using an Agilent 4284A LCR 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 was calculated using the equation,

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

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

3. Results and Discussion
In figure 1and 2 the photograph shows the grown crystals in (a) gel media (b)brushite + 0.5 ml green tea

Fig 1: Brushite crystals grown in gel media

Fig 2: Brushite + 0.5 ml green tea

It was observed that the incorporation of green tea caused a decrease in the number of grown brushite crystals and their average size. By carefully observing the shape, size, and approximate number of crystals obtained, a graph is drawn to explain the promotery/inhibitory effect of the pure and the green tea and illustrated in figure 3.

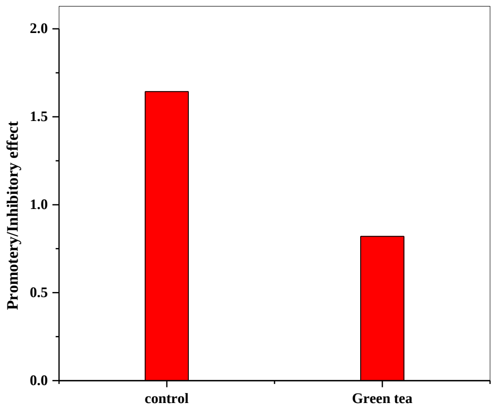

Fig 3: Promotery/Inhibitory effect of brushite crystals

3.1. Powder X-Ray Diffraction
The powder X-ray diffraction for both pure and green tea added crystal is shown in Fig 4 & 5respectively.The brushite crystal crystallizes in the monoclinic structure and the lattice parameters are in good agreement with a reference pattern [JCPDS no 72-0713).The PXRD pattern of green tea added crystals shows shift in the peak positions, change in peak intensity and appearance of new peaks at an angles 53.57º, 58.82º, 64.18º and 67.90º respectively.This finding also suggests the chances of incorporation of green tea extracts within the framework of CHPD.

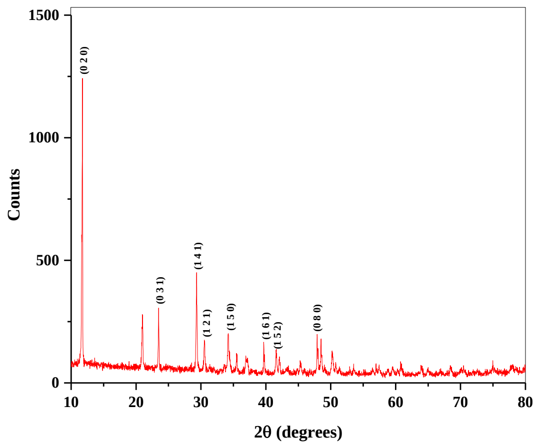

Fig 4: PXRD pattern of pure brushite crystals

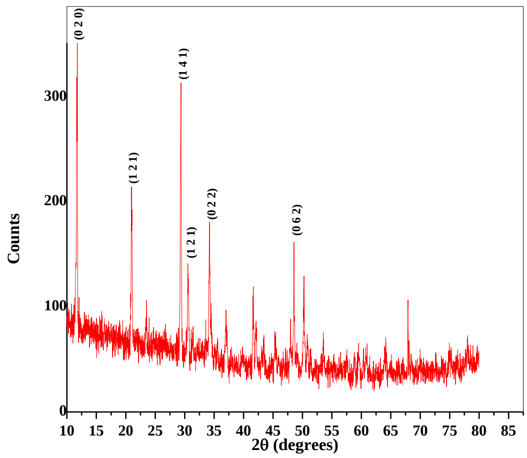

Fig 5: PXRD pattern of green tea added brushite crystals

Table 1:Lattice parameters and unit cell volume

Samples	Unit cell parameters			Unit cell volume V (Å)^3
	a (Å)	b (Å)	c (Å)	
Pure Brushite	5.2125	15.1781	5.605	443.444
0.5ml of Green tea	5.2076	15.1717	5.5819	441.015

The crystallite size of the CHPD crystals were calculated by using Scherrer equation,

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

Where D is the crystallite size, K is the constant and usually taken as 0.89, λ is the wavelength of X-ray radiation, β is the full width at half maximum value and θ is the Bragg diffraction angle.From the PXRD spectrum the crystallite size of undoped CHPD is found to be 49.26 nm and green tea incorporated CHPD it is found as 43.19 nm.

3.2. UV-Visible Spectral Analysis

The UV-Vis absorption spectra were recorded for all the grown samples using UV-Vis Double Beam Spectrophotometer 2201 in the wavelength range 200-600 nm. The UV-Visible absorption spectrum of all the grown samples crystals is shown in figures (6-7).

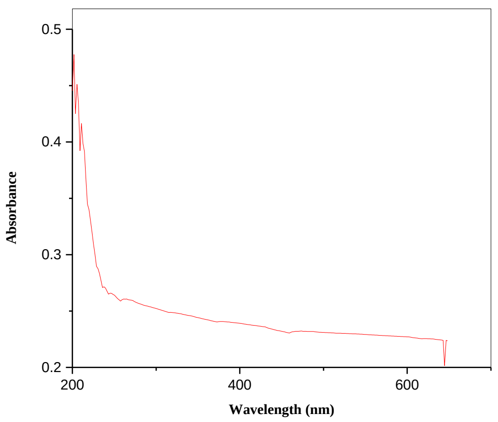

Fig 6: UV-Vis spectra of pure brushite crystals

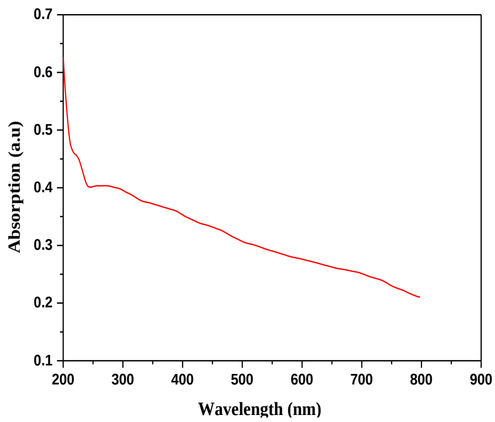

Fig 7: UV-Vis spectra of green tea added brushite crystals

Fig 6 shows the recorded optical absorption spectrum of grown pure brushite crystal in which characteristic absorption band occurs below 210 nm. It was found that there is no significant absorption in the entire spectrum of study for both the pure and Green tea added brushite, which means that the grown crystals were transparent in the entire UV-Visible region.The band gap for the pure brushite crystal was found to be 9.16 eV. For the green tea added brushite crystals, the band gap energy decreases.

3.3. Dielectric studies

Materials, which are electric insulators or in which an electric field can be sustained with a minimum dissipation power are known as dielectric materials. As the dipolar nature of calcium phosphate is because both the elemental constituent of kidney stone being ionic and polar the dielectric properties also give an idea about how these materials behave with the applied alternating current of various frequencies.

The value of dielectric constant (ε_r) of a material is usually composed of four contributions; which are from electronic, ionic, dipolar and spacecharge polarizations. However, all these may be active in low frequencyregion. The nature of variation of dielectric constant with frequency indicateswhich contribution is prevailing. The space - charge contribution depends onthe purity and perfection of crystal. The dipolar orientational effect can be sometimes seen upto 1010 Hz. The ionic and electronic polarizations alwaysexist below 1013 Hz [12]

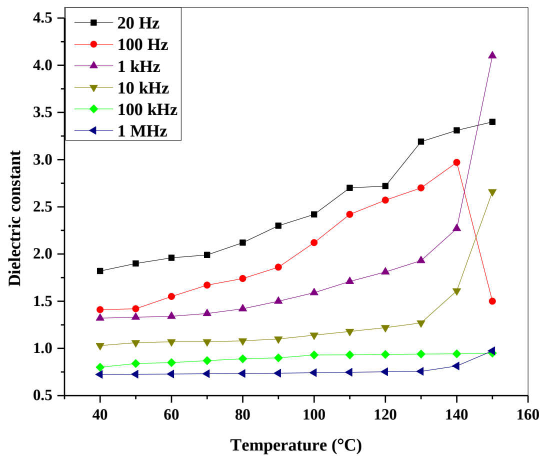

Fig 8: Dielectric constant of pure brushite crystals

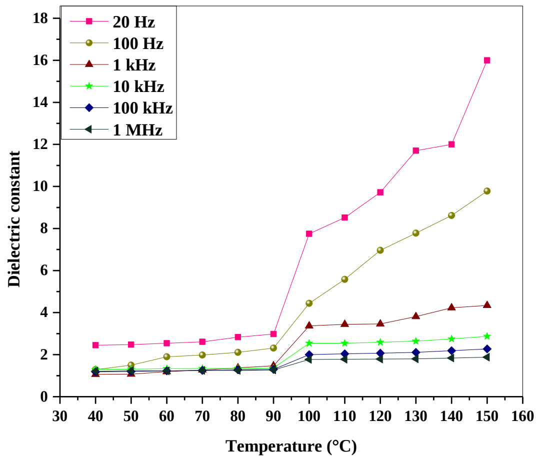

Fig 9: Dielectric constant of green tea added brushite crystals

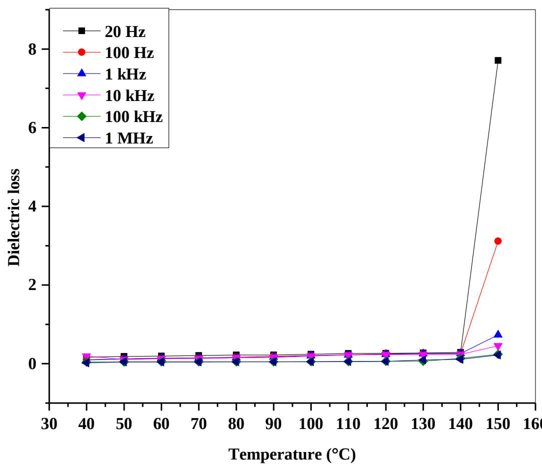

Fig 10: Dielectric loss of pure brushite crystals

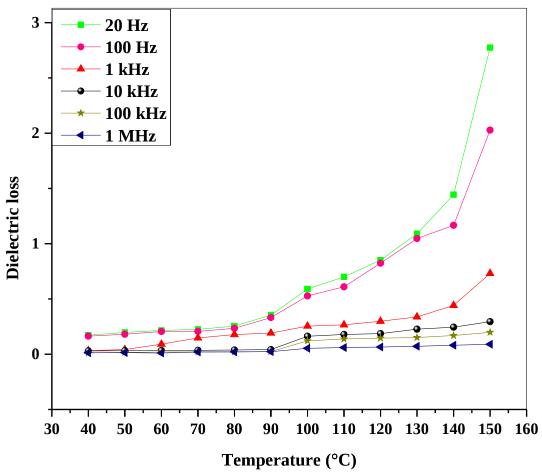

Fig 11: Dielectric loss of green tea added brushite crystals

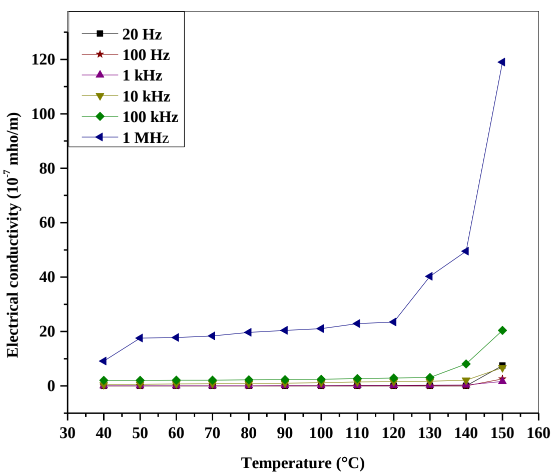

Fig12: AC Electrical conductivity of pure brushite crystals

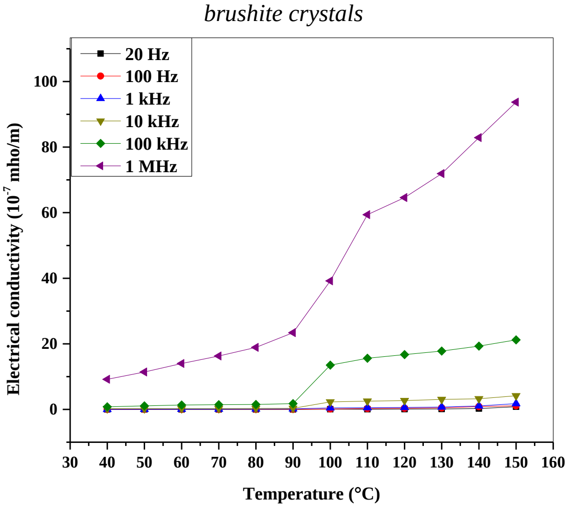

Fig 13: AC Electrical conductivity of green tea added brushite crystals

From the graph it was observed that, at each particular temperature the dielectric constant shows decrease in its value as the frequency increases. The decrease in dielectric constant with increase infrequency is a normal dielectric behaviour and can be explained onthe basis of polarization mechanism.The mechanisms of polarization have varying time response capability to an applied field frequency, and the net contribution of polarization to the dielectric constant is therefore frequency dependent.The reduction in the values of dielectric constant with frequency may be due to the space charge which cannot sustain and comply with the electric field. The variation of dielectric loss (tanδ) versus frequency suggests that dielectric loss decreases as frequency increases.
It was found that ac conductivity increased with the increasing value of frequency of applied field. The ac conductivity is expected due to the hopping of protons through vacancies in hydrogen bonding.

4. Conclusion

The effect of green tea on the growth of CHPD was evaluated by using single diffusion gel growth technique. Gel method is the most versatile and simple technique to study the growth, inhibition of urinary stones under in vitro conditions. The present study revealed that the green tea is very effective to inhibit the growth of CHPD crystal growth under in vitro gel conditions. The PXRD analysis shows the decrease in crystallite size for green tea added CHPD crystals.The frequency and temperature dependence of the dielectric parameters were different for undoped and green tea added CHPD crystals and throws light on the polarization mechanisms in these crystals. It is expected that as the amount of green tea applied increased, the size of the crystals became considerably reduced and finally form less stable kidney stones that break up more easily.

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