Volume 11, Number 2, 2018. pp. 91-99

Jordan Journal of Physics

ARTICLE

Growth Inhibition of Natural Foods on Calcium Hydrogen Phosphate Dihydrate Crystals*

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Received on: 22/5/2017 *Accepted on: 10/12/2017*

Abstract: A large number of people are suffering from health problems due to urinary stones. The prevalence of urinary stones is increasing and approximately 12,000 patients are admitted in hospitals every year due to this condition. Brushite [$CaHPO_4{\cdot}2H_2O$] or calcium hydrogen phosphate dihydrate (CHPD), which is known as urinary crystal, is a stable form of calcium phosphate. This study aims to investigate the influence of natural foods on the formation of calcium hydrogen phosphate (CHPD) crystals to elucidate the inhibitory effect of urinary stone formation from a different perspective. Natural foods used in this study are *Solanum lycopersicum* (Tomato), *Daucus carota* (Carrot) and *Vitis vinifera* (Grapes). Tomato is the edible fruit of *Solanum lycopersicum*, which belongs to the nightshade family, *Solanaceae*. Grapes are a fruit, botanically a berry, of the deciduous woody vines of the flowering plant genus vitis. The carrot is a biennial plant in the umbellifer family *Apiaceae*. The effects of natural foods, the *in-vitro* crystallization and growth inhibition of calcium hydrogen phosphate dihydrate crystals are studied using single diffusion gel growth technique. The grown crystals were characterized by total mass determination, X-ray diffraction, UV-Vis and Dielectric studies. The crystalline size is reduced for natural foods added CHPD crystals compared with pure CHPD. The grown crystals are more transparent and of a size of around 53 and 49 nm. Some variations in dielectric properties are observed due to the addition of fruit extracts.
Keywords: Natural foods, Growth inhibition, CHPD crystals.

Introduction

Many people suffer from problems resulting from urinary stones. The presence of amorphous calcium phosphate is a common finding in urinary sediments and it is the most commonly encountered crystal material in urine [1- 2]. The disease frequency is on the rise due to life style and dietary habits [3-4]. Calcium-containing stones are the most common variety of urinary stones, comprising about 75% of all urinary calculi, which are found in the form of pure calcium oxalate (50%), calcium phosphate (5%) or a mixture of both (45%). Some of the oxalates are found in either pure or in mixed form with phosphate and reported with uric acid or ammonium urates [5-6]. Also, calcium phosphate is present in urinary calculi as either apatite [$Ca_{10}(PO_4)_6(OH)_2$] or brushite [$CaHPO_4.2H_2O$] [7-8]. Urinary stones are characterized by their high recurrence rate, if patients are not treated appropriately. Recent approaches to the treatment of urinary stones make use of Laser Lithotripsy, Percutaneous Nephrolithotomy and Extra-corporeal Shockwave Lithotripsy [9-10]. Though they provide immediate clearance of urinary stones, they cause long-term side effects [11-13]. Patients suffering from brushite

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*"6th National Seminar on Advances in Materials Science NSAMS-2017, held on March 2, 3-2017 at the Department of Physics, Manonmaniam Sundaranar University, Tirunelveli-627 012, Tamilnadu, India".

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 system of medicine, *Solanum lycopersicum* (tomato), *Daucas carota* (carrot) and *Vitis vinifera* (grapes) were used for the treatment of various human ailments, like kidney stone, diabetes, urinary tract infection and immune system. Most of the previous papers deal with the drug therapy for the inhibition of urinary stones, but the juice therapy on the growth inhibition of these crystals was not reported. Considering the above facts, the current study is carried out to find out the inhibiting effect of fruit extracts, which are used as natural foods; *viz*., tomato, carrot and grapes, on brushite crystals.

The growth of crystals in a gel medium has attracted the attention of many investigators [14-16]. This is because gel method is the most versatile and simple technique for growing urinary crystals [17–19], where the gel acts as an inert and ideal medium during the growth of many crystalline compounds and *in-vitro* biomolecules [20].

In this work, we study the inhibitory effect of natural foods on the growth of calcium hydrogen phosphate dihydrate crystals using single diffusion gel growth technique.

Experimental

The high quality AR grade chemicals from Merck are used further without purification. Glass test tubes of 25 mm diameter and 150 mm length were used as crystal growth apparatus. Sodium meta silicate ($Na_2SiO_3 .9H_2O$) solution with a specific gravity of 1.03g/cc is mixed with orthophosphoric acid in an appropriate amount, so that the desired value of the pH could be set for the mixture. After setting the gel, an aqueous solution of calcium chloride and calcium acetate of a particular molarity (1M) is gently poured on the set gels in test tubes by using a pipette without damaging the gel surface. After pouring the supernatant solution, the test tubes were capped with airtight stopples. The following reaction is expected to take place, leading to the formation of calcium hydrogen phosphate dihydrate crystals:

$$CaCl_2.2H_2O + CaC_4H_6O_4 + H_3PO_4 \longrightarrow CaHPO_4.2H_2O + CaCO_3 + HCl$$

In order to understand the inhibitory effect of natural foods on the growth of CHPD crystals, extracts of 0.5ml of tomato, 0.5ml of carrot and 0.5ml of grapes are mixed with calcium chloride and calcium acetate and the crystals are grown as before. The grown crystals are elongated, plate- like and star shaped. The crystal growth was complete in about 25 days. The grown crystals are carefully removed and collected in a clean petri dish and then harvested by removing the gel using distilled water.

Powder X-ray diffraction data is collected using an automated X-ray diffractometer with CuK_α radiation (λ = 1.54060 Å). The UV-visible spectrum of the sample is recorded in the wave number range of 200-600 nm using UV-Vis Double Beam Spectrophotometer 2201.

Dielectric studies are carried out with the help of Agilent 4284A LCR meter at different temperatures and at different frequencies ranging from 20 Hz to 1 MHz.

The dielectric constant of the crystals is 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 is calculated using the equation:

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

where 'ω' is the angular frequency and 'tan δ' is the loss tangent.

Results and Discussion

Growth

Calcium hydrogen phosphate dihydrate (brushite) crystals were successfully grown using the single diffusion gel growth technique. The addition of supernatant solution; namely, calcium acetate and calcium chloride over the set gel enabled the diffusion of Ca^{2+} into the gel, which reacts with PO_4^{3+}, resulting in the appearance of precipitate instantaneously at the interface between the gel and the supernatant solution. After 4 to 5 hours just below the precipitate, circular white discs, commonly called Liesegang rings, were observed. Tiny plate- like brushite crystals started growing just below the Liesegang rings in about 4 to 8 days and also inside the gel medium. The crystal growth was complete in about 25days. Figs. 1 and 2 show good quality star and plate- shaped crystals grown in gel media.

FIG. 1. Brushite crystals grown in gel media.

FIG. 2. Brushite + juices (0.5ml tomato + 0.5 ml carrot + 0.5 ml grapes).

The incorporation of fruit solution caused a decrease in the number of grown brushite crystals and their average size. By carefully observing the yield of crystals per test tube, we present in Fig. 3 the promotery/inhibitory effect of the pure and the fruit extracts.

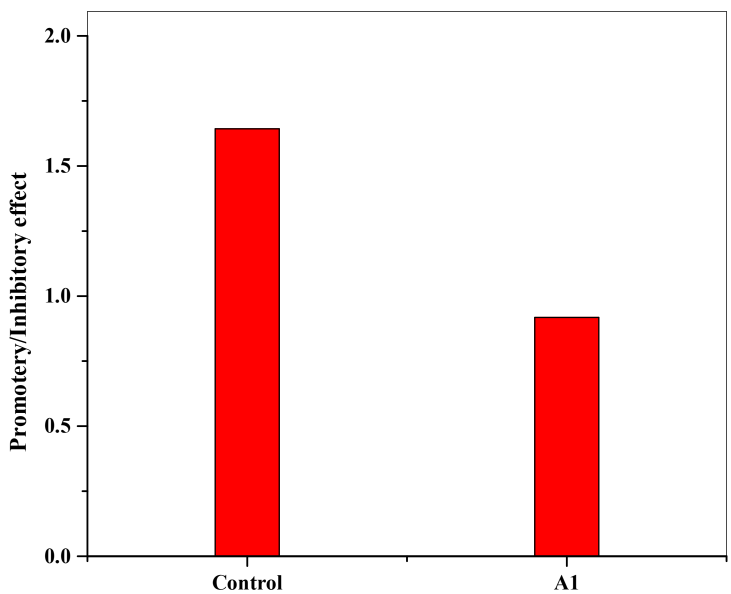
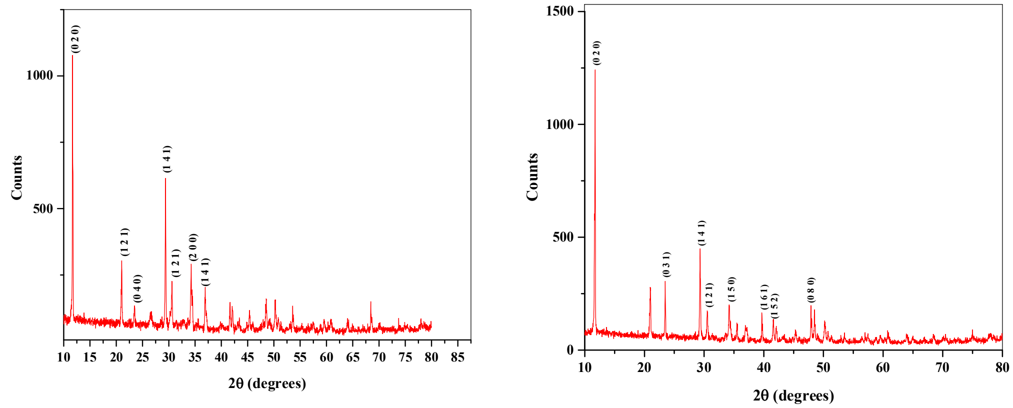


FIG. 3. Promotery/inhibitory effect of brushite crystals.

In Fig. 3 above, A_1 represents the fruit solution added brushite crystals.

Powder X-ray Diffraction

The X-ray diffraction patterns of the grown crystals (shown in Figs. 1 and 2) are shown in Fig. 4 (a and b), respectively. Brushite is crystallized in the monoclinic crystal system. The crystallographic parameters are in good agreement with the literature values (JCPDS data) [72-0713]. The highly resolved peak occurs at specific 2θ Bragg angles in the crystals, indicating the crystalline nature of the grown crystals. It is also observed that the peak values shift towards higher angles, indicating the chances of incorporation of additional ions available in the used fruit extracts within the framework of CHPD.



(a) (b)

FIG. 4. PXRD patterns of (a) pure CHPD crystals; (b) fruit extracts added CHPD 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 CHPD	5.2125	15.1781	5.605	443.444
Fruit extracts added CHPD (A_1)	5.1958	15.1580	5.9963	472.177

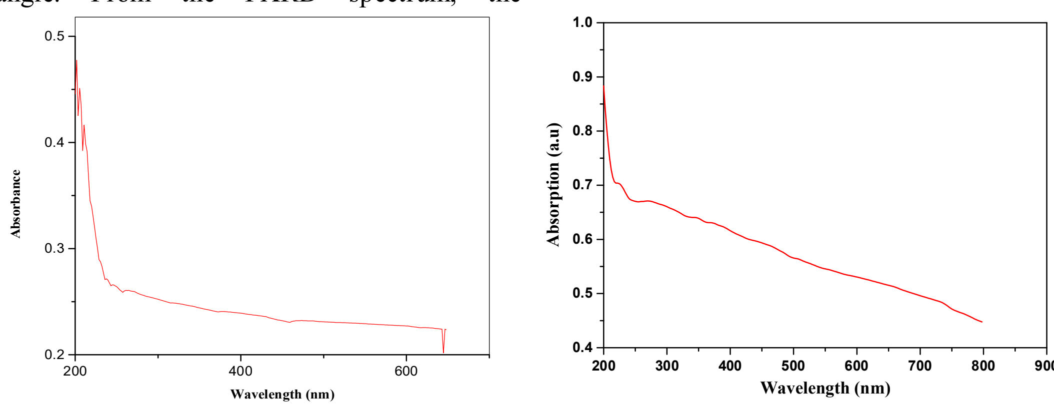
The crystalline size of the CHPD crystals was calculated by using Scherrer equation:

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

where D is the crystalline size, K is a constant 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 crystalline size of undoped CHPD was found to be 53.72 nm, while for fruit extracts incorporated CHPD, it was found to be 49.51 nm.

UV-Vis Spectral Analysis

The UV-visible absorption spectra recorded for both grown samples (Figs. 1 and 2) are shown in Fig. 5 (a and b), respectively.



(a) (b)

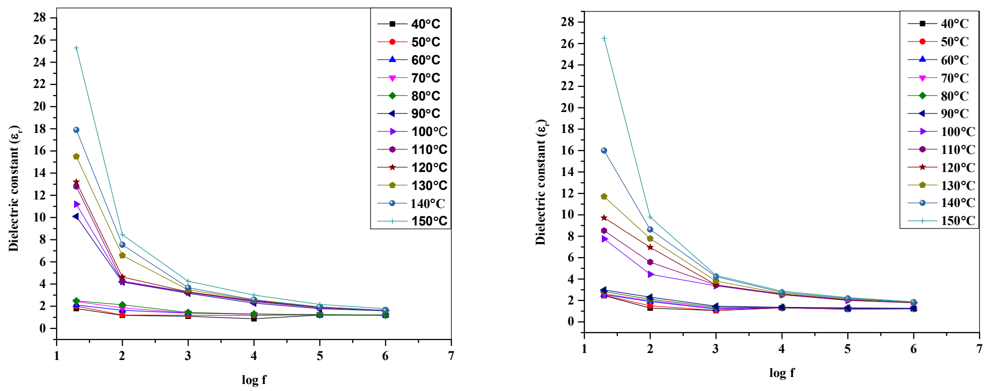
FIG 5. UV-vis spectra of (a) pure CHPD crystals; (b) fruit solution added CHPD crystals.

The UV-visible spectrum gives information about the structure of the molecules. The ultraviolet and visible light absorption involves promotion of electrons from the ground state to higher energy state. Careful analysis of the spectrum revealed no significant absorption in the entire spectrum for both pure and fruit extracts added brushite. This indicates that the grown crystals were transparent in the entire spectral range between 370 and 600 nm. The band gap for the brushite crystals grown in the control system was found to be 5.48 eV. For the fruit extracts added brushite crystals, the band gap energy decreased to 5.38 eV.

Dielectric Studies

Dielectric Constant

Figs. 6 (a and b) show the variation of dielectric constant with frequency for various temperatures ranging from 40ºC - 150ºC for pure CHPD and fruit extracts added CHPD crystals. It is noticed that for all temperatures, the dielectric constant decreases as the frequency increases for pure CHPD crystals. Similar behaviour is observed for fruit extracts added crystals as well. The dielectric constant of a material is usually composed of four components of polarization; namely, electronic polarization, orientation polarization, ionic polarization and space charge polarization. The electronic and ionic polarizations are independent of temperature, whereas orientation polarization and space charge polarization are temperature-dependent.



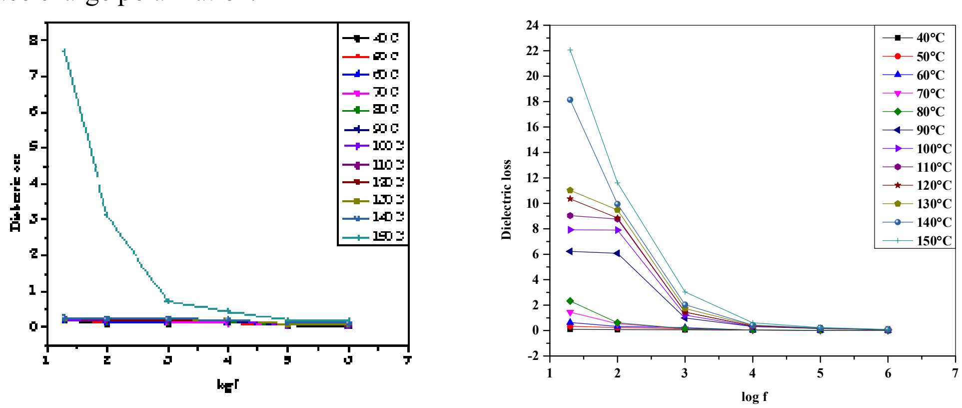
(a) (b)

FIG. 6. The variation of dielectric constant with temperature for different frequencies for (a) pure CHPD crystals; (b) fruit extracts added CHPD crystals.

From Fig. 6 (a and b), it is clear that at low frequency (20Hz) and at low temperature (40ºC), all the four types of polarization are active, but when the frequency increases, the variation of dielectric constant shows which contribution is prevailing. When the temperature is above 100ºC and the frequency is very low, say 20Hz, the dielectric constant is maximum. This is because of the contribution of space charge polarization. As we further increase the frequency, the dielectric constant decreases. This is because of the decrease in the space charge polarization.

Dielectric Loss

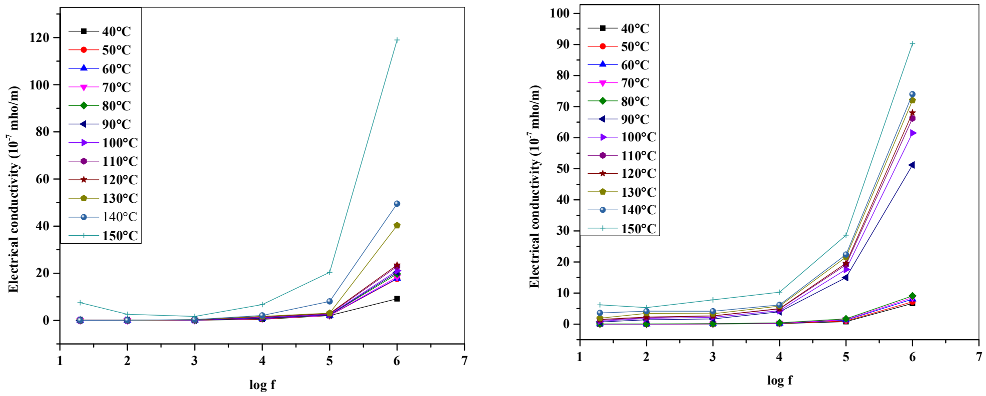
The variation of dielectric loss (tan δ) with frequency at different temperatures for pure CHPD and fruit extracts added CHPD is shown in Fig. 7 (a and b), respectively. The amount of energy dissipated by the material when it is subjected to external fields is called dielectric loss. It was found from Fig. 7 that the dielectric loss increases with the increasing value of applied field. The high value of dielectric loss at high frequencies indicates the low power dissipation in the crystals.



(a) (b)

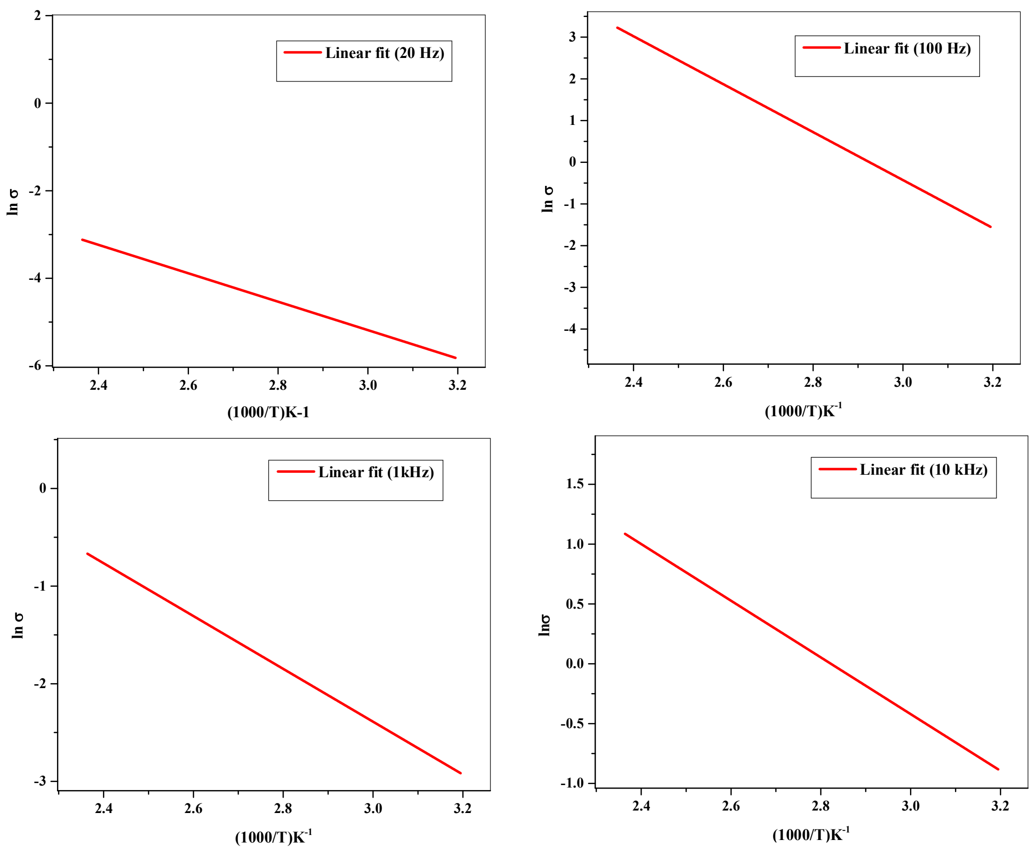
FIG. 7. The variation of dielectric constant with temperature for different frequencies for (a) pure CHPD crystals; (b) fruit extracts added CHPD crystals

AC conductivity & Activation energy



(a) (b)

FIG. 8. The variation of a.c. conductivity with temperature for different frequencies for (a) pure CHPD crystals; (b) fruit extracts added CHPD crystals.



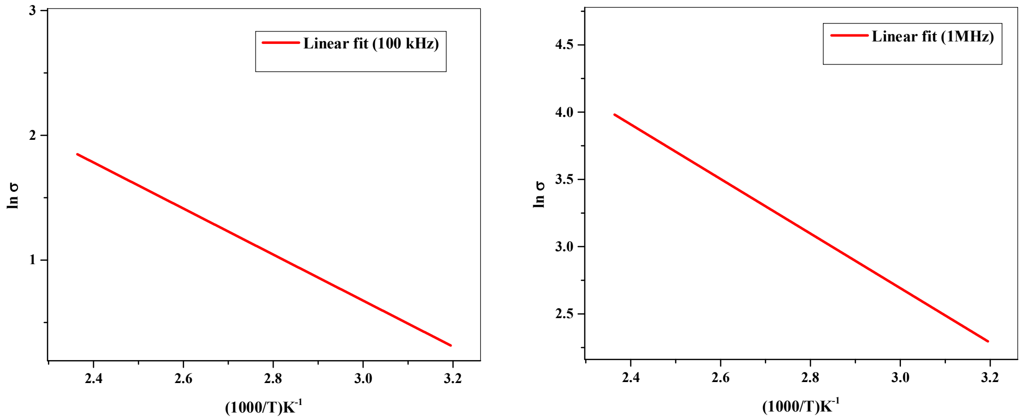


FIG. 9. Plots between lnσ_{ac} and 1000/T for pure CHPD crystals.

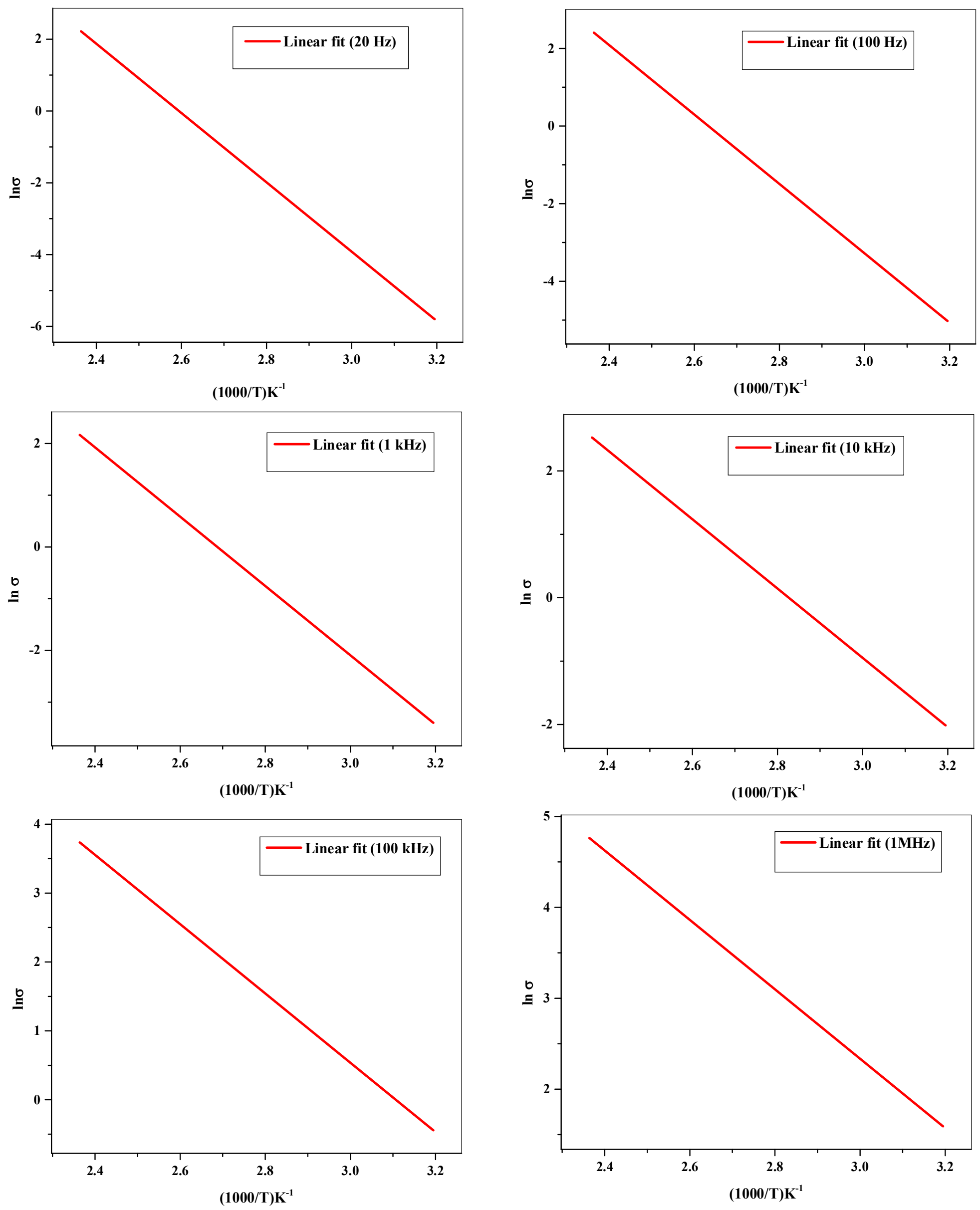


FIG. 10. Plot between ln$\sigma_{ac}$and 1000/T for fruit extracts added CHPD crystals.

TABLE 2. Values of activation energy (E_g) at 20 Hz, 100 Hz,1 kHz, 10 kHz, 100 kHz and 1MHz for pure CHPD and fruit extracts added CHPD crystals.

Samples	20 Hz (eV)	100 Hz (eV)	1 kHz (eV)	10 kHz (eV)	100 kHz (eV)	1 MHz (eV)
Pure CHPD	0.27986	0.49542	0.23309	0.20411	0.15896	0.17482
Fruit extracts added CHPD	0.83197	0.77041	0.57718	0.47100	0.43350	0.32894

The a.c. conductivity (σ_{ac}) of a material depends on the dielectric nature of the sample. There is a strong correlation between the temperature dependence and the frequency response of electrical conductivity of materials. In the present case, the frequency-independent conductivity is observed upto 100 kHz, whereas above this frequency it exhibits frequency dispersion. 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 behavior, such that $\sigma ac = A\omega^s$ [9].

The conductivity values at different temperatures are represented by Arrhenius plots.

Arrhenius equation for conductivity is expressed as follows:

$$\sigma = \sigma_0 \exp (-E_g/KT) \quad (1)$$

where $\sigma_0$is the pre-exponential factor, E_g is the activation energy, K is the Boltzmann's constant and T is the absolute temperature.

Taking the natural logarithm of both sides, the above equation becomes:

$$\ln(\sigma) = \ln(\sigma_0)-E_g/KT \quad (2)$$

Graph of ln(σ) *vs*. 1000/T is plotted to calculate the value of activation energy.

From Table 2, it is found that the juice-incorporated CHPD crystals exhibit high activation energy and the value of activation energy decreases with increasing frequency. Non-systematic variation of E_g values was observed for pure CHPD crystals.The value of activation energy given in Table 2 shows that the charge carriers are ionic [21]. Thus, the results obtained indicate that the electrical properties of CHPD crystals could be tuned significantly by addition of fruit extracts.

Conclusion

Brushite crystals are grown by single diffusion gel growth technique. Powder XRD studies show that the grown brushite crystals exhibit monoclinic structure and the lattice parameters are in good agreement with the literature values [JCPDS no. 72-0713). Reduction in crystalline size with addition of fruit extracts is observed as compared with pure CHPD crystals. The UV-vis analysis showed good transparency of the grown crystals. Electrical parameters, such as dielectric constant and dielectric loss, were found to decrease with increasing the value of frequency of the applied field and a.c. conductivity increased with increasing the value of frequency of the applied field. Activation energy values of the samples have been evaluated. The incorporation of fruit extracts was exhibited very well in the activation energy values with comparison to pure CHPD crystals. This study shows that fruit extracts prepared from natural foods have good inhibitory effect on the growth of the brushite urinary stone crystals considered.

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