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ORIGINAL ARTICLE

Growth and Spectral Characterization of Calcium Oxalate Crystals

I V Priadharshini[1], **R Selvaraju**[2]*

1 Department of Physics, Annamalai University, Annamalai Nagar, Tamil Nadu, India
2 Department of Engineering Physics (FEAT), Annamalai University, Annamalai Nagar, Tamil Nadu, India

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* **Corresponding author**.

drselvarajufeatau@gmail.com

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Abstract

Backgrounds/Objectives: Urolithiasis, characterized by the presence of calculi in the urinary tract, involves the formation of stones in any part of the urinary system, including the kidneys, ureters, bladder, or urethra. Calcium oxalate contributes to the formation of kidney stones (urolithiasis) in the urinary tract. This is a mineral belonging to the oxalate group and is found naturally in humans, animals and plants. These stones are responsible for the majority of urinary calculi. The present work examines the crystal formation of calcium oxalate using silica gel in laboratory conditions and its characterization by spectroscopical methods. Methods: Calcium oxalate crystals were grown from the sodium metasilicate using the single diffusion gel growth technique. The harvested crystals were analysed using X-Ray Diffraction (XRD), FT-IR Spectroscopy, UV-Visible spectrophotometer, Field Emission Scanning Microscopy (FE-SEM), and Thermogravimetric Differential Thermal Analysis (TG-DTA) studies. The dielectric constant and dielectric loss as a frequency function were measured for the grown crystal. Findings: The gel growth technique is extremely effective for producing high-quality crystals in a controlled and reliable way. The prevention of calcium oxalate kidney stones could be beneficial for society. Novelty: This study integrates the multispectral approach in the growth of crystals and helps in producing a focus for the prevention or dissolution of the urinary-type stones.

Keywords: Calcium oxalate; Gel method; Spectral Characterisation

1 Introduction

Urolithiasis, also known as kidney stones, arises when the kidneys produce crystalline deposits in the urinary tract, referred to as stones. Stone dimensions are typically categorized using one or two measurements; they are classified into sizes up to 5 mm, 5 to 10 mm, 10 to 20 mm, and those exceeding 20 mm in the largest diameter. Stones can be classified based on their anatomical location: the upper, middle, or lower calyx; the renal pelvis; the upper, medium-sized, or distal ureter; and the urinary bladder(1). Urinary stones form when minerals in the urine aggregate and proliferate instead of being diluted and eliminated from the body. They assume various shapes and are composed of different materials. The composition of stones affects subsequent diagnostic and treatment decisions. Various substances often constitute stones. They are the causes of urological diseases that affect both men and women. Up to 50% of urological diseases affect men more than women, and men have a higher risk of developing kidney stones compared to women.

The consumption of proteins, salt, and oxalate-rich foods such as peanuts, chocolates, and potatoes contributes to the formation of kidney stones (2). Dehydration is a significant factor that impacts the function of the kidneys. Additional factors, including genetics, age, lifestyle, medications, and environmental influences, significantly contribute to the formation of urinary calculi. Identifying the type of kidney stone can aid in determining the reason and reducing the risk of recurrence. Hence, more studies have started to analyze the urolith morphology and chemical composition using SEM and optical microscopy (3).

Numerous factors affect the growth of urinary calculi. Calcium-containing stones are the predominant type, making up approximately 75 percent of all urinary calculi, with pure calcium oxalate representing 50 percent of this category. The remaining stones consist of either calcium phosphate (5%) or a combination of both (45%). Various mineral metabolisms play an essential role in the development of urinary stones or calculi. Compared to other types of kidney stones, calcium oxalate stones affect more than 80% of humans (4). Calcium oxalate stones exist in three distinct forms: calcium oxalate monohydrate (COM), which is also known as whewellite, calcium oxalate dihydrate (COD), commonly referred to as weddellite, and calcium oxalate trihydrate (COT), also known as caoxite. Some stones are made of magnesium ammonium phosphate, uric acid, or cystine, and having too much of these substances in the body can lead to the creation of calcium oxalate minerals (5). Here, the calcium oxalate is observed due to calcium excretions associated with the absence of crystallization inhibitory capacity, while the calcium oxalate dihydrate has a high level of calcium excretion in urine with normal excretion of citrate and urinary PH>6. The creation of calcium oxalate (CaOx) stones in urine is affected by how much CaOx is present and the balance of things that help or prevent its formation (6).

Many researchers crystallize the pure and doped crystals to find out innovative materials for various purposes. The substantial influx of information regarding crystal development in gels has been organized into three primary segments, each offering distinct advantages that will be discussed in the following sections: (i) crystal growth through reaction; (ii) crystallization via the complex dilution method; and (iii) crystal growth through reduced solubility. Gel preparation can be done by five different methods, likely silica, gelatin, agar, clay, and polyacrylamide, where the silica gel method improves the crystallinity of the product in the gel (7). Crystals are commonly observed during nearly all developmental phases. The method is suitable for large-scale crystal fabrication. By varying the growing conditions, one can obtain crystals with various morphologies and sizes. By the single diffusion gel method, calcium oxalate crystals are grown and characterized because this technique is simple and effective for growing urinary-type crystals (8).

Calcium oxalate crystals are a primary component of kidney stones, and study investigates their origin and potential adverse effects caused by their accumulation in kidney tissue, along with the potential long-term health implications. Research studies often examine the formation of calcium oxalate crystals inside the body, taking into account factors such as urine composition, pH levels, and the influence of other molecules that can either promote or prevent crystallization. The gel growth technique is extremely effective for producing high-quality crystals in a controlled and reliable way, especially for complex crystal structures or biological systems. It helps understanding of the mechanics behind crystal formation in biological systems, including bone and urinary calculi. This study integrates a multispectral approach to crystal growth and aids in developing strategies for the prevention or dissolution of urinary-type stones. The present work examines the crystal formation of calcium oxalate using silica gel in laboratory conditions and its characterization by analytical methods. The optical, thermal, and dielectric studies of grown calcium oxalate crystals are conducted.

2 Materials and Methods

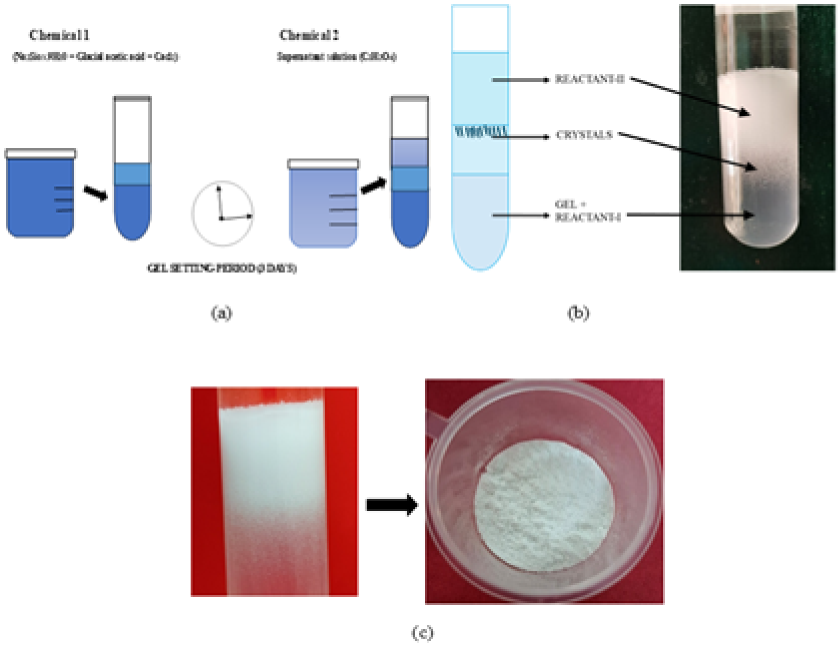
Chemicals of the AR grade were utilized in the investigation of the formation of calcium oxalate crystals. These chemicals included Sodium metasilicate (SMS), Glacial acetic acid, Calcium chloride, Oxalic acid, and Distilled water. Using borosilicate glass test tubes with a length of 20cm and a diameter of 2.5mm, the complete crystal formation procedure took place in an uncontaminated and highly controlled atmosphere.

2.1 Gelification and the formation of calcium oxalate crystals in gel substrates

The gel was made by mixing sodium metasilicate ($Na_2SiO_3.9H_2O$) powder in double-distilled water and shaking it well. After filtering the solution, it was stored in a clean flask (9). This solution was then combined with glacial acetic acid while maintaining a pH between 6.0 and 6.5. Additionally, it was mixed with a specific gravity of 1.04 grams per cubic centimeter. Following this, the sodium metasilicate solution was mixed with a 0.7M calcium chloride ($CaCl_2$) solution, and the mixture was allowed to set at room temperature for seventy-two hours. After the gel had been set, a liquid oxalic acid (CaC_2O_4) solution with a concentration of 0.6M was poured slowly and carefully around the borders of the test tubes over the already set gel. The test tubes are tightly covered with corks, kept still, and vibration-free (10). The chemical reaction that occurred was as follows:

$$CaCl_2 \cdot H_2O + C_2H_2O_4 \longrightarrow CaC_2O_4 \cdot H_2O + 2HCl$$

As Figure 1(a) shows, the schematic diagram of the preparation of crystals in the gel growth method. After 168 hours (7 days), fine crystals were formed in the test tubes as shown in Figure 1(b). The formation of crystals at the centre of the gel region, and some of them are located on the surface of the test tube (11). Crystals were then collected from the test tubes after completing 21 days, and the grown crystals were harvested and placed on filter paper for weighing, as shown in Figure 1(c). Table 1 lists the ideal conditions for calcium oxalate.



(a)

(b)

(c)

Fig 1. (a) Schematic diagram of the preparation of calcium oxalate crystals in the gel growth method (b) A calcium oxalate crystal forming within the gel (c) The harvested calcium oxalate crystals

Table 1. The Optimum Conditions for the Formation of Calcium Oxalate

S. No	Parameters	Optimum Values
1	Density of sodium metasilicate	1.04gm/cm^{-3}
2	PH of gel	Between 6.0 and 6.5
3	Concentration of calcium chloride	0.7M
4	Concentration of oxalic acid	0.6M
5	Gel setting period	3 days
6	Gel aging	1 month
7	Period of growth	21 days
8	Temperature	Room temperature
9	Quality	Smooth white deposition (tiny crystals)
10	Lattice type	Monoclinic

2.2 Experimental details

The FTIR spectrum of the calcium oxalate crystals was recorded by the KBr pellet technique using a Perkin-Elmer 180 spectrometer between 400 and 4000 cm^{-1} and is available at Centralized Instrumentation, Science Laboratory, Department of Physics, St. Joseph's College (Autonomous), Tiruchirappalli. Powder X-ray diffraction of the sample is carried out by Expert-Pro with CuKα radiation of λ=1.54Å, which is available in Alagappa University, Department of Physics, Karaikudi. The UV-visible absorption spectrum of the sample was studied between 200-800nm using the UV-2400PC series, which can be found in the Science Laboratory, Department of Physics, St. Joseph's College (Autonomous), Tiruchirappalli. FE-SEM was recorded by using Carl Zeiss-Sigma 300 at the range of 1 1μm-200nm, which is available in the Centralized Instrumentation and Service Laboratory (CISL), Department of Physics, Annamalai University. The crystal samples are subjected to TG-DTA in the range of room temperature to 1400°C at 10 or 20°C/min by using Netzsch-STA 449 F3. Jupiter is available in the centralized instrumentation and service laboratory (CISL) in the Department of Physics at Annamalai University. Dielectric studies were analyzed using the HIOKI LCR METER IM 3536 at room temperature, which is available at Physaac Research Laboratory, Department of Physics, Arul Anandar College, Madurai.

3 Result and Discussion

A single gel diffusion approach successfully forms calcium oxalate crystals during the growth process. The analytical methods, such as PXRD, FT-IR, UV-Visible, TG-DTA, FE-SEM, and dielectric studies, were used to study the gel-grown crystals. These techniques are used to characterize the grown crystals. The grown crystal is identified as monoclinic, as evidenced by the earlier work(1). The sharp peaks from the PXRD analysis of calcium oxalate crystals proved their crystalline nature. The FT-IR spectrum identifies the functional groups in the grown crystal(2). Calcium oxalate crystals absorb the two shoulder peaks at 212 and 248nm in UV-visible studies, indicating the material's activity(3). The subsequent step comprises the breakdown of calcium oxalate at temperatures ranging from 360°C to 600°C, ultimately emitting carbon dioxide and carbon monoxide gases(4). The results indicate that there are no exothermic peaks in the DTA curve. Thermal stresses at the nucleation site induce plastic deformation, which leads to the observed irregular diamond-shaped dislocations(5). The dielectric studies indicate a slight decrease in frequency associated with low defect levels(6).

3.1 PXRD analysis

The XRD spectrum of the crystal powder sample is shown in Figure 2. The sample was scanned at a rate of one degree per minute, ranging from 10° to 50°. The crystal powder from the X-ray analysis reveals peaks that correspond to the title compound. JCPDS (14-0789) for calcium oxalate hydrate yields the related d values and their intensity, as well as the h, k, and l parameters(12). From the observation, the peak value of 2θ had high intensity at 14.9376, followed by a second intensity 24.5505, and then 30.1608, suggesting that the crystals are composed of calcium oxalate. The grown crystal is monoclinic, as evidenced by the earlier work(13). The sharp peaks obtained from the formed calcium oxalate crystals provide evidence of their crystalline nature(14). Table 2 lists the data of powder X-ray diffraction for calcium oxalate crystals.

3.2 FT-IR analysis

Figure 3 shows a recorded FTIR spectrum for the crystal. The spectrum identifies the vibrations of different functional groups in the grown crystal(15). The many vibrational functional groups confirm the synthesized crystal(16). Figure 3 explores the information bands associated with the distinct vibrations of crystals. The calcium oxalate crystal sample exhibited a stretching vibration of OH at 3491 cm^{-1}, which corresponds with symmetric stretching, and at 3434 cm^{-1}, which is attributed to asymmetric stretching. Both of these stretching vibrations are attributable to stretching. Furthermore, a strong absorption band at 3282 cm^{-1} shows that the stretching of the OH group is caused by intermolecular hydrogen bonding. As the band at 3065 cm^{-1} shows, the C-H stretching pattern occurs when atoms of carbon and hydrogen move closer together and subsequently move further away(17). The symmetric C=O stretching vibrations are the cause of the sharp absorption band that may be found at 1622 cm^{-1}. 1622 cm^{-1}, 1384 cm^{-1}, and 1319 cm^{-1} are the three frequencies at which asymmetric C-O stretching bands are formed. Furthermore, the C-O symmetric stretching band of calcium oxalate crystal may be formed at a frequency of 950 cm^{-1}. Crystals of calcium oxalate can be recognized by a strong absorption band at 885 cm^{-1}, which is linked to the C-C stretching vibration. The vibration indicates the presence of two carboxylate anions. The sharp peak at 782 cm^{-1} is where the O-C=O band is found. The spectrum indicates this band, characteristic of the bending vibrations of water molecules. With two additional peaks at 596 cm^{-1} and 517 cm^{-1}, which are attributed to metal-oxygen bonds(18), and the peak attributed at 420 cm^{-1} is assigned to metal-metal-oxygen bonds. The FTIR spectrum indicates the formation of calcium oxalate crystals since it confirms the presence of O-H stretching,

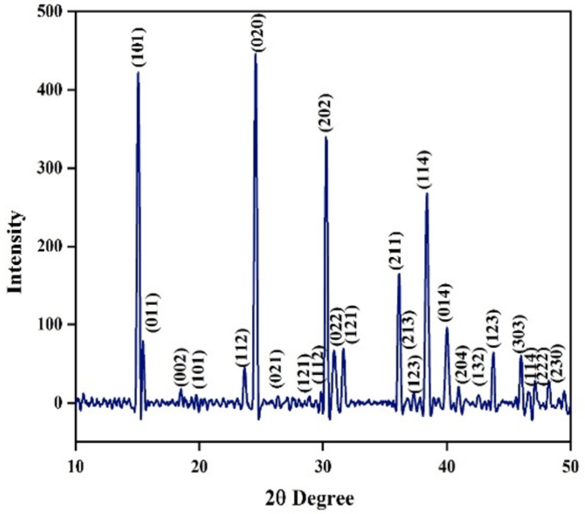


Fig 2. PXRD spectrum of calcium oxalate (CaOx)

Table 2. Powder X-ray diffraction of calcium oxalate crystals

Standard values		**Observed values**	
2θ	**I/I0**	**2θ**	**I/I0**
14.927	100	14.9376	100
15.29	60	15.3396	20
19.58	40	19.6634	6
23.516	40	23.5602	11
24.366	100	24.5505	96
28.68	20	28.7817	3
30.084	80	30.1608	72
30.818	20	30.9108	13
31.44	40	31.5434	15
37.136	20	37.3073	4
38.302	60	38.3833	43
39.782	40	39.9969	13
40.796	40	40.9567	6
42.38	20	42.5461	4
43.56	60	43.7409	23
45.837	60	45.9858	14
46.509	40	46.6273	8
47.201	10	47.1168	13
48.076	40	48.2444	10

C-O stretching, C-H stretching, O-C-O bonds of water of hydration, and metal-oxygen bonds. Table 3 gives assignments of absorption bands in the FT-IR spectrum of calcium oxalate.

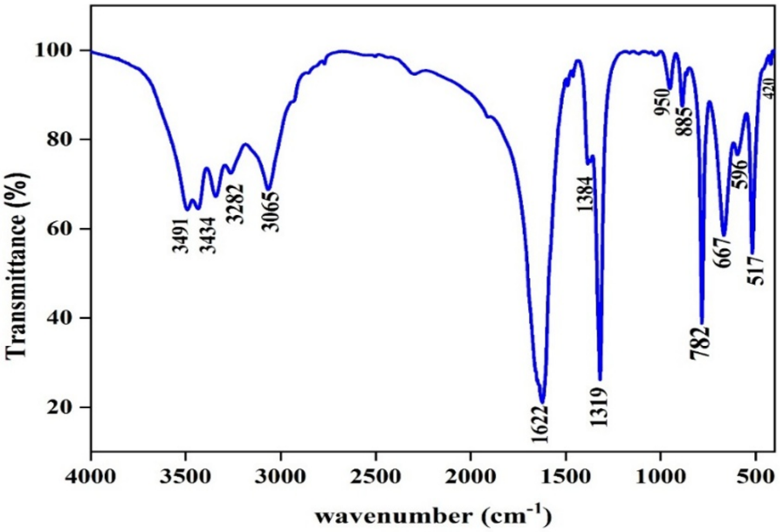


Fig 3. FT-IR spectrum of gel-grown calcium oxalate (CaOx)

Table 3. Assignments of absorption bands in the FT-IR spectrum of calcium oxalate

Wavenumber (cm^{-1})	Tentative Assignment
3491	OH stretching
3434	OH stretching
3282	OH stretching
3065	C-H stretching
1622	C-O Stretching
1384	C-O Stretching
1319	C-O Stretching
950	Vibration of C-O
885	Vibration of C-O
782	O-C-O stretching
667	OH bending
596	Metal oxygen bond
517	Metal oxygen bond
420	Metal oxygen bond

3.3 UV-visible analysis

The changes in the molecule's electrons allowed us to measure how the crystal absorbs light from 200 to 1100 nm in the UV range of the UV-visible spectrum. The optical absorbance frequencies are compared with literature data. The observed absorbance occurs in the ultraviolet (UV) spectrum between 200 and 800 nm, as indicated by the spectrum in Figure 4. The peaks are observed at 212nm, 248 nm and 298nm demonstrating the material's UV activity(19). The resultant three shoulder peaks may be due to some impurities or a change in atmosphere. Investigating the ultraviolet-visible spectrum is essential to determine a material's transparency, as it determines its optical activity(20). Furthermore, various applications involving NLO can leverage the transparent nature of the earlier-mentioned crystals in the UV-visible range. It has been concluded that the advantageous properties of materials with NLO activity include a lower cut-off wavelength and the optical transmission window range (21).

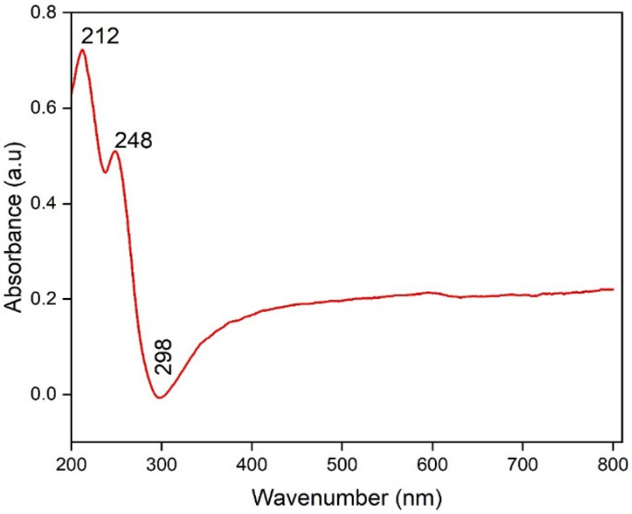


Fig 4. UV-Visible spectrum of calcium oxalate (CaOx)

3.4 TG-DTA analysis

The thermal stability of the sample was studied in a nitrogen environment using thermogravimetric (TG) and differential thermal analysis (DTA) (22). Figure 5 presents the results of the TG-DTA analysis. The TGA shows two significant processes. The formation of calcium oxalate (CaOx) occurs during the initial phase, driven by water evaporation, starting at 40°C and continuing until reaching 350°C (23). The subsequent step involves the decomposition of calcium oxalate at temperatures ranging from 360°C to 600°C, ultimately resulting in the release of carbon dioxide and carbon monoxide gases (24). The mass loss occurs between 29°C and 159°C at 4%, the second mass loss occurs between 159°C and 209°C at 6%, and the final loss occurs between 209°C and 439°C at 20%. The differential thermal analysis (DTA) curve shows two cooling points at 230°C and 490°C, which relate to the breakdown of calcium oxalate. The DTA curve did not exhibit any exothermic peaks (25).

3.5 FE-SEM analysis

SEM is used to obtain information about surface topography, grain size, and crystal composition (26). Electronic devices necessitate flawless crystals for their multiple purposes. The analysis of plastic deformation and crystal morphology is essential. Figure 6 provides an illustration of the FE-SEM. The FESEM image showed irregular diamond-shaped crystals with smooth surfaces in four different sizes: 1μm, 500nm, 300nm, and 200nm, taken at different magnifications of 10kx, 20kx, 30kx, and 40kx. These images are captured at various magnifications. Thermal stresses at the nucleation site induce plastic deformation, causing the observed irregular diamond-shaped dislocations. Figure 6 presents an illustration of the Field Emission Scanning Electron spectrum in its physical form (27). The title compound has substantial elements, including carbon, oxygen, potassium, and calcium, resulting in a smoother crystal surface characterized by irregular diamond-shaped formations. This transformation occurs as a result of the presence of these elements (28).

3.6 Dielectric analysis

The dielectric loss and dielectric constant of the crystal were measured to study its electrical behavior at room temperature. The dielectric constant (ϵr) versus the logarithm of the applied frequency is shown in Figure 7. The dielectric constant shows a high value at one point and then decreases with frequency, which is indicative of polarization effects. The large value of the dielectric constant (ϵr) at low frequencies is attributed to the presence of space charge polarization, which depends on the purity and

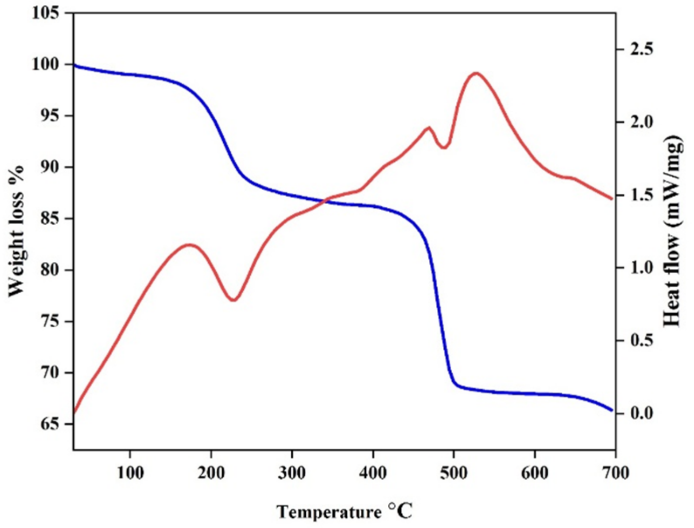


Fig 5. TG-DTA curve of calcium oxalate (CaOx)

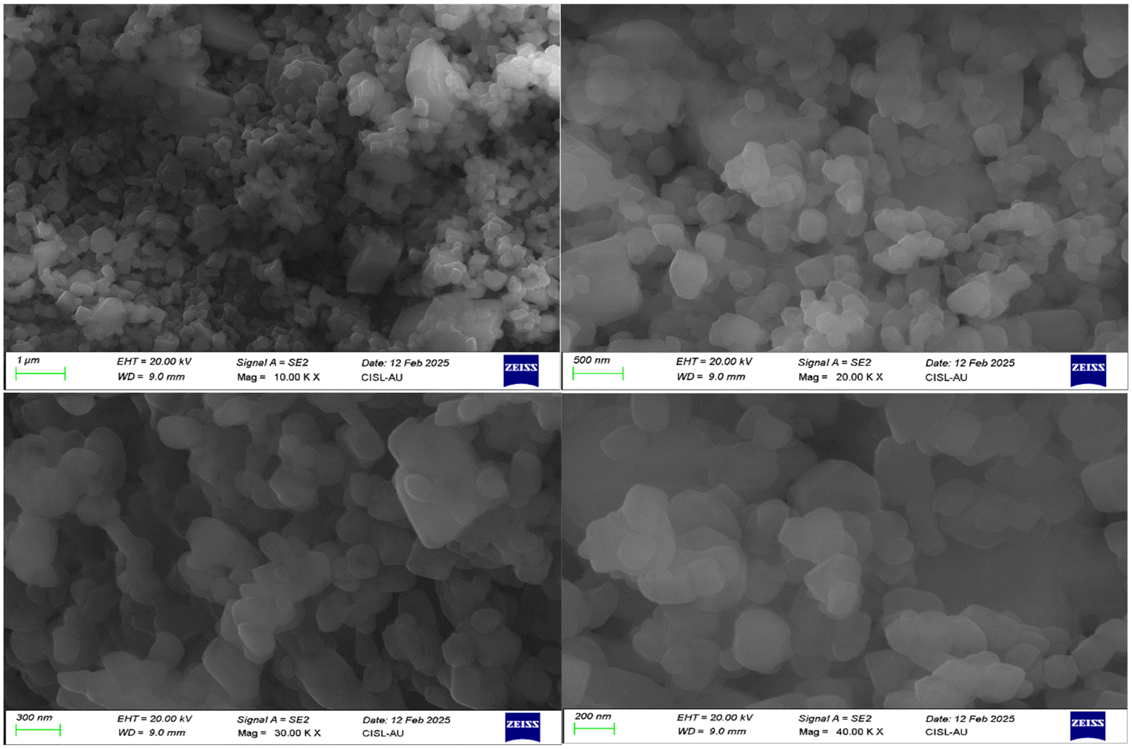


Fig 6. FESEM images of calcium oxalate (CaOx)

perfection of the sample. Figure 7 illustrates that the sample's minimal dielectric loss in the high-frequency region indicates a low impurity density.(29).

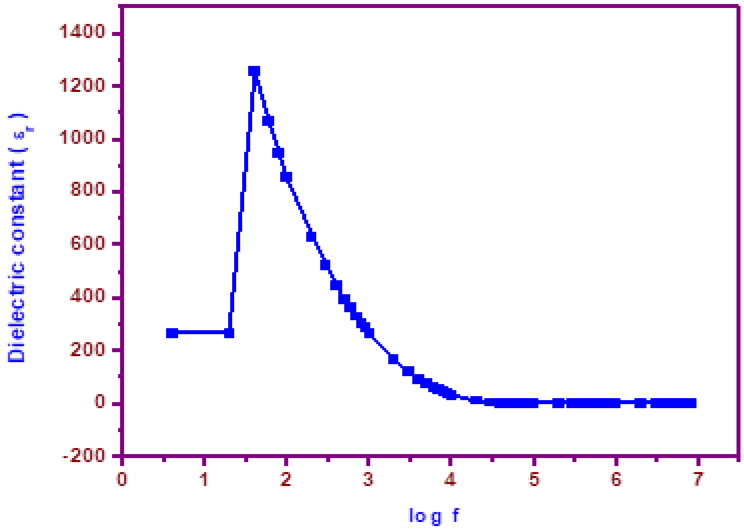


Fig 7. Dielectric constant (ϵr) with log frequency of calcium oxalate (CaOx)

Dielectric loss is the material's absorption of the applied electric field's energy. The dielectric loss in the low-frequency region is not frequency-dependent. Figure 8 shows that as the frequency increases, the dielectric loss decreases. At high frequency, the dielectric constant is almost zero. At high frequencies, the dielectric constant drops due to electric polarization effects(30). The analysis of the dielectric constant and dielectric loss plotted against log frequency illustrates the loss reduction with frequency, indicating low defect levels.

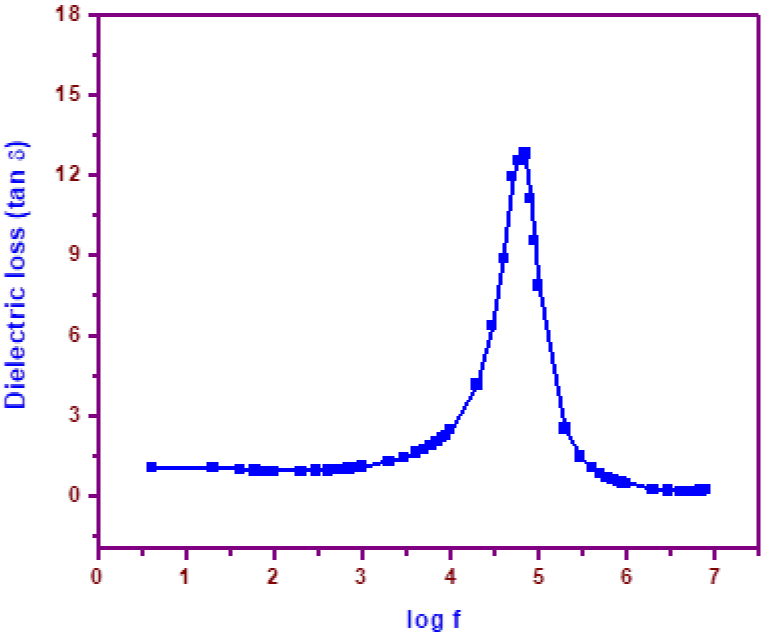


Fig 8. Dielectric loss (tan δ) with log frequency of calcium oxalate (CaOx)

4 Conclusion

This investigation offers a comprehensive examination of the methods employed in the synthesis and collection of calcium oxalate crystals through the use of a singular diffusion gel growth method. The chemical composition of the grown crystals is determined using a number of spectroscopic techniques. These methodologies encompass ultraviolet visible spectroscopy (UV-visible), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), thermo gravimetric differential thermal analysis (TG-DTA), field emission scanning electron microscopy (FE-SEM), and dielectric analysis. The crystals that grow possess a monoclinic structure. The X-ray diffraction technique is used to determine the lattice parameters and crystalline structure. The FT-IR spectra verify the presence of the functional groups. The crystal's absorption and cutoff wavelength are investigated using the Ultraviolet-Visible (UV-VIS) method. The thermal stability and melting point of the crystal are ascertained through the use of TG-DTA. The calcium oxalate crystals are not precisely planar and exhibit an irregular diamond-like shape, as evidenced by FESEM analysis. The crystal's dielectric properties have been investigated, and it has been determined that it has a low dielectric constant and dielectric loss at high frequencies. It is important to determine whether pharmaceuticals or natural substances interact with calcium oxalate crystals in order to advance the field of kidney stone prevention. It may be beneficial to know about crystal development, shape, and size in order to facilitate the production of materials with the desired properties. The research is advancing as a result of the utilization of specific plant extracts to dissolve calcium oxalate crystals. These findings suggest that society may benefit from preventing the formation of calcium oxalate kidney stones.

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