

Some critical aspects of FT-IR, TGA, powder XRD, EDAX and SEM studies of calcium oxalate urinary calculi

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Urinary calculi constitute one of the oldest afflictions of humans as well as animals, which are occurring globally. The calculi vary in shape, size and composition, which influence their clinical course. They are usually of the mixed-type with varying percentages of the ingredients. In medical management of urinary calculi, either the nature of calculi is to be known or the exact composition of calculi is required. In the present study, two selected calculi were recovered after surgery from two different patients for detailed examination and investigated by using Fourier-Transform infrared spectroscopy (FT-IR), thermo-gravimetric analysis (TGA), powder X-ray diffraction (XRD), scanning electron microscopy and energy dispersive analysis of X-rays (EDAX) techniques. The study demonstrated that the nature of urinary calculi and presence of major phase in mixed calculi could be identified by FT-IR, TGA and powder XRD, however, the exact content of various elements could be found by EDAX only.

Keywords: Urinary calculi, Characterization, Calcium oxalate monohydrate, Phosphate impurities

The urinary calculi or kidney stones are solid masses of sediments formed in some part of the urinary tract. They result from various factors, such as metabolic abnormalities, nutritional factors, bacterial infection and environmental factors¹ and their prevalence has increased in last two decades^{2,3}. The urinary calculi are mainly oxalate and phosphate types and usually occur as mixed-type; however, some rare components like apatite, uric acid and organic matrix are also found⁴. Around 12% of population in the European Union suffers at least one process related to urolithiasis⁵. In India, 12% of population is expected to have urinary stones, out of which 50% may end up with loss of kidneys or renal damage. Moreover, nearly 15% of the population of north India suffers from kidney stones⁶.

A spectrum of pediatric urolithiasis in Western India has been reported⁷. In this study, 1,211 pediatric patients were treated, out of that 77 (6.4%) pediatric cases and the commonest age group was 6-10 yrs (55.8%), showing high prevalence of urinary calculi. In the Western India, particularly Saurashtra region of Gujarat state comes under the region of very warm climate, low rain fall and increased salinity of ground water, is considered as a high urinary stone disease prone area. The study conducted in Saurashtra region on 3405 treated patients, out of which 37.33% were urinary calculi patients and the commonest age group was 21 to 40 yrs, i.e., the prime working age⁸. A high incidence of urinary stones in coastal villages of Junagadh district in Saurashtra region, Gujarat, has been studied in terms the poor quality of drinking water with high dissolved salts and the socio-economic impact⁹ due to large number of patients.

In the present investigation, two different calcium oxalate types of urinary calculi, recovered after surgery, were analyzed by Fourier-Transform infrared (FT-IR) spectroscopy, thermo gravimetric analysis (TGA), powder X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive analysis of X-ray (EDAX), in order to determine composition, impurities and the type of other phases present in the calculi.

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Abbreviations: COM, calcium oxalate mono-hydrate; EDAX, energy dispersive analysis of X-rays; FT-IR, Fourier-transform infrared spectroscopy; LIPS, laser induced plasma spectroscopy; SEM, scanning electron microscopy; TGA, thermo gravimetric analysis; XRD, X-ray diffraction.

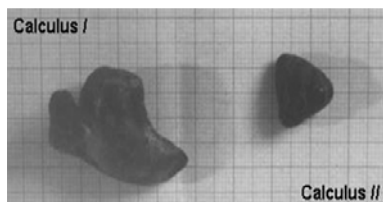


Fig. 1—Calculus I and II after surgical removal

Materials and Methods

Two oxalate types of urinary calculi (calculus I and II, Fig. 1) were recovered after surgery from two different patients (by JGB) for detailed examination. The calculus I (size approx. 20.3 mm × 15.3 mm × 13.3 mm) was recovered from the right renal pelvis of 45 yrs old female patient, while calculus II (size approx. 6.68 mm × 5.56 mm × 4.48 mm) was recovered from 73 yrs old male patient from the right side mid-ureteric stone with hydro-ureter and hydro-nephrosis.

FT-IR spectroscopy

The FT-IR spectra of powdered samples of urinary calculi were recorded on Nicolet Magna IR 550 Series II FTIR spectrometer in KBr medium in the range from 400 to 4000 cm^{-1} to confirm the presence of various functional groups in the samples.

Thermo-gravimetry analysis (TGA)

The TGA was carried out on powdered urinary calculi from room temperature to 950°C with the heating rate of 15°C/min in an atmosphere of nitrogen using $\alpha\text{-Al}_2\text{O}_3$ as standard reference which helps in identifying the amount of water of hydration and thus to study the thermal stability of the samples.

Powder XRD

To find out the crystal structure of the sample, the powder XRD patterns were analyzed by PW1710 based diffractometer with $\text{Cu-K}\alpha$ radiation by using PC APD diffraction software. The crystal structure was determined by software VISER.

SEM and EDAX

To study identification based on textural ground, SEM and EDAX investigations were carried out using Oxford micro analysis (Group LEICA S 440 Model 7060). For SEM, the maximum magnification of 10 kX was used. The EDAX had determination area of 10 mm × 10 mm having ATW 1 Window with resolution from 5.9 to 138 keV.

Results and Discussion

There are different etiologies and theories proposed for the urinary calculi formation¹⁰. Although analysis

of stones by various techniques and the identification of type of stones have been reported, these techniques are only helpful in the medical management for the particular type of calculus. Thus, in the present work calculi were characterized by FT-IR, TGA, powder X-ray diffraction and SEM studies.

FT-IR Study

FT-IR spectroscopic method has been used to investigate urinary calculi¹¹ and has found calcium oxalate as the main constituent with the presence of phases of hydroxyl and carbon apatite; however, it sometimes fails in identifying calcium oxalate and struvite¹². Several upgraded infra-red (IR) techniques have also been used to analyze the urinary calculi, for instance, regression analysis program enables better quantization of stone components¹³, the use of golden gate reflection diamond attenuates total reflection sample holder, and artificial neural network along with the computer library facilitate the FT-IR spectral interpretation of urinary calculus¹⁴. In FT-IR study of urinary calculi procured from south India, the vibrational frequencies for calcium oxalate monohydrate and dihydrate, uric acid and struvite have been reported^{15,16}. IR and Raman spectroscopy have also been used for analysis of urinary calculi^{17,18}. In the present study, the conventional FT-IR spectral study was used without any further instrumental and software modifications to analyze the urinary calculi.

The FT-IR spectrum of urinary calculus I is depicted in Fig. 2a. The absorption at 3484 cm^{-1} was due to inter molecular and weak O-H bond due to water of crystallization, while absorption at 3338 cm^{-1} and 3436 cm^{-1} was attributed to symmetric and asymmetric O-H stretching vibrations, respectively. The absorption at 3256 and 3060 cm^{-1} was due to O-H-O stretching vibrations and at 1907 cm^{-1} was due to C=O and -O-CO-O- stretching vibrations, whereas at 1620 cm^{-1} and 1317 cm^{-1} was attributed to the asymmetric and symmetric stretching vibrations of CO_2 , respectively. The absorption at 780 cm^{-1} was due to the presence of metal-oxygen or δ (O-C=O) or P-O-P asymmetric stretching vibration and the vibrations at 662 cm^{-1} and 517 cm^{-1} were attributed to rocking of CO_2 or due to (HO) P=O.

The FT-IR spectrum of urinary calculus II is shown in Fig. 2B. The absorption at 3433 cm^{-1} was due to inter molecular and weak O-H bond due to the water of crystallization, at 1620 cm^{-1} was due to the

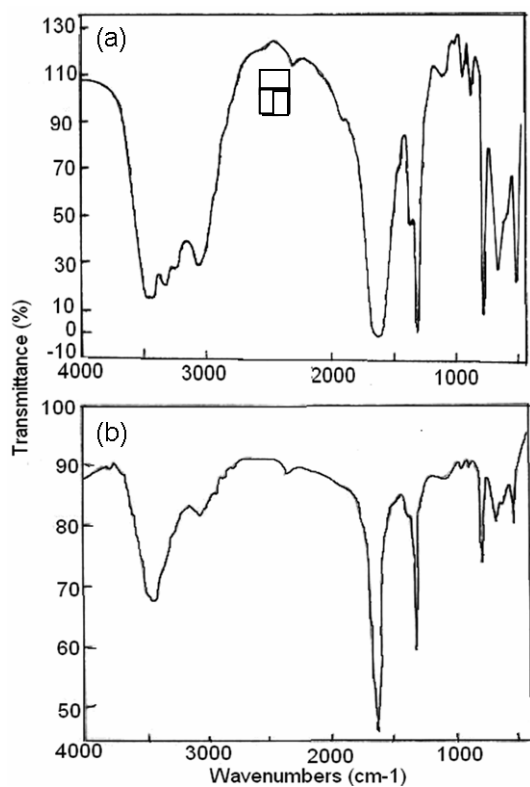


Fig. 2—FTIR spectrum of calculus I (a) and II (b)

asymmetrical stretching vibration of CO_2 and at 1317 cm^{-1} was attributed to symmetrical stretching vibration of CO_2 . Alike in calculus I, the absorption at 780 cm^{-1} was due to presence of metal-oxygen or $\delta(\text{O-C=O})$ or P-O-P asymmetric stretching vibration and the absorption at 662 and 517 cm^{-1} was attributed to rocking of CO_2 or due to $(\text{HO})\text{P=O}$.

It can be noticed from Fig. 2a and b that both FTIR spectra were similar and they exhibited the same type of urinary calculi. When the urinary calculi are of mixed nature, the FT-IR spectra differ from that of pure calcium oxalate, calcium hydrogen phosphate dihydrate (CHPD-brushite)¹⁹ and ammonium magnesium phosphate hexa-hydrate (struvite)⁷. It is difficult to identify the exact composition of urinary calculi (whether calcium oxalate, brushite or struvite in mixed forms) by FT-IR spectroscopic study, as the asymmetric stretching vibration of P-O-P as well as $(\text{HO})\text{P=O}$ vibration due to PO_4^{3-} give rise to absorptions at 1025 cm^{-1} and 870 cm^{-1} range and 500 cm^{-1} to 580 cm^{-1} variable range, respectively. These ranges also overlap for metal-oxygen vibration as well as $\delta(\text{O-C=O}) + \nu(\text{M-O})$ and rocking of CO_2 . Due to overlapping nature of characteristic absorptions of calcium oxalate, brushite and struvite in the above ranges, it is difficult to identify the

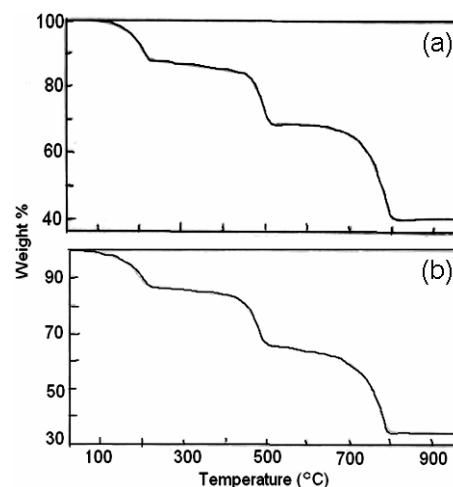


Fig. 3—Thermogram of calculus I (a) and II (b)

composition all phases in distinct manner using alone FT-IR spectroscopy.

Earlier, Hideo²⁰ performed the qualitative analysis of calcium oxalate stone by IR spectroscopy and ascribed the typical absorption bands of apatite in the region $1100\text{--}1000\text{ cm}^{-1}$ and of calcium oxalate at 1320 cm^{-1} , 920 cm^{-1} , 770 cm^{-1} and 520 cm^{-1} . Both calculi I and II showed the characteristic calcium oxalate absorptions at 1317 cm^{-1} , 780 cm^{-1} and 517 cm^{-1} . Thus, FT-IR spectroscopy could provide the characteristic signature of functional groups and bonds present, but did not provide exact amount of water molecules. Hence, thermo-gravimetry was performed to ascertain the exact amount of water molecules in the calculi.

TGA study

Earlier, the contents of weddellite (calcium oxalate dihydrate) and whewellite (calcium oxalate monohydrate) have also been confirmed by TGA²⁰. As the difficulties appear in the differentiation of hydrates of calcium oxalates, the thermal techniques have been employed²¹. Thermal study has also been carried out on various calculi obtained from south India^{15,16}. Many workers have also studied the properties of calculi by employing thermal analysis²²⁻²⁴. The thermo-balance and FT-IR coupled through a heat transfer line also helps in analyzing urinary calculi, particularly in the case of complex mixtures²⁵.

The thermogram of calculus I (Fig. 3a) indicated thermal instability by losing water molecules at 50°C and being converted into anhydrous form around 260°C . The dehydrated sample then decomposed into CaCO_3 around 590°C by the evolution of CO and

Table 1—Thermal decomposition of calculus I and II

Calculus I			Calculus II		
Temp. °C	Theoretical% wt loss	Practical% wt loss	Temp. °C	Theoretical% wt loss	Practical% wt loss
Room temp.	100	100	Room temp.	100	100
260	87.68	87.83	270	87.68	86.42
590	68.51	68.69	640	68.51	62.65
900	38.38	41.38	880	38.38	34.35

finally into CaO at 900°C by further evolution of CO₂. The thermo-gram of urinary calculus II (Fig. 3b) showed that the calculus started giving up water molecules from 50°C and became anhydrous around 270°C and, thereafter, on further heating decomposed into CaCO₃ around 640°C and ultimately into CaO at 880°C.

Table 1 shows that the observed weight loss values were very close to the predicted theoretical values. From the calculations, it was found that one water molecule was associated with both the calculi. Further comparing the thermo-grams of urinary calculi with that of calcium oxalate monohydrate crystal²⁹, it was confirmed that both calculi were of calcium oxalate mono-hydrate (COM) type with other impurities present. However, estimation of impurities was not possible directly from TGA results.

Powder X-ray diffraction study

X-ray diffraction pattern has been used to characterize and identify different urinary calculi²⁷ and also to determine the proportional rate of the particular crystalline components forming the calculus. Also, it provides a well-organized reliable facility, which can give clinicians reliable results within a few hours^{28,29}. The X-ray diffraction is reported to give almost 100% correct results for brushite and apatite and shows no ambiguity in results of analysis, as compared to FT-IR analysis of struvite and calcium oxalate¹². Based on the type of urinary calculi, clinicians administer drugs to treat patients either with thiazide diuretics, allopurinol, potassium citrate or with any other drug³⁰.

The urinary calculi in humans are mainly composed of calcium oxalate (as monohydrate and dihydrate) and calcium phosphate and cystine. Majority of calculi do contain more than 10% of one of these or more combinations of these crystalline components, including the trace amounts of other urinary substances, which presumably are

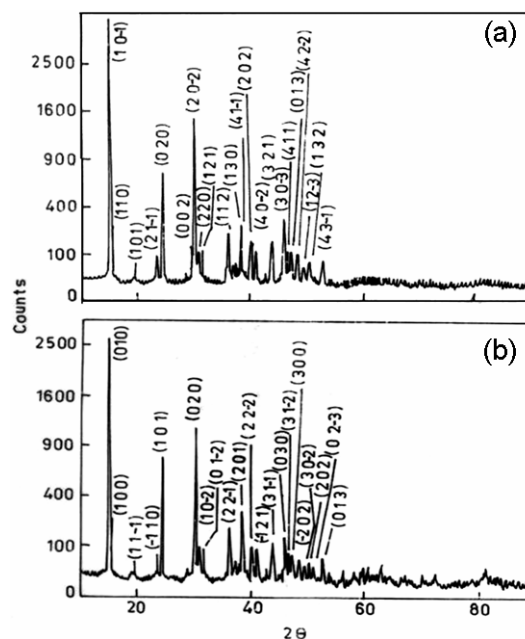


Fig. 4—Powder XRD patterns of calculus I (a) and II (b)

mechanically incorporated in the growing calculus. This has lead combining crystal-optical and X-ray diffraction methods to investigate several urinary calculi with their core and shell separately³¹. It suggests that the cores are mostly mono-mineralic and peripheral parts mostly bi-mineralic.

In one of the study, different constituents of urinary calculi have been identified by powder XRD³². In addition, multi-phases of hydroxyl and carbonate apatites have been detected¹¹. Moreover, the different phases of calcium oxalate in calculi collected from north India have been identified by XRD analysis of samples³³. Also, difficulty in the identification of different components separately in mixed urinary calculi is reported³³. Notwithstanding, the study has indicated that XRD analysis alone can not be regarded as a routine technique for quantitative characterization of urinary calculi, but semi-quantitative XRD analysis supplemented by accurate quantitative elemental analysis data is more suitable for precise determination of true stone composition³⁴.

An attempt was made to assign the reflections in the powder XRD patterns of both calculi to either oxalate or phosphate radicals or calcium atoms by comparing them with the XRD patterns of COM, brushite and struvite crystals. Figure 4 (a and b) shows the XRD patterns for urinary calculus-I and II, respectively. Table 2 shows the comparison of 2θ and d values of calculus I and II with COM, CHPD and struvite crystals, where reflections 1, 6, 7, 9, 11 and

Table 2—Comparison of 2θ and d values of calculus I and II with COM, CHPD and struvite crystals

Sr. no. of peaks	COM crystals		Calculus I		Calculus II		CHPD crystals		Struvite crystals	
	$2\theta^\circ$	$d \text{ \AA}$	$2\theta^\circ$	$d \text{ \AA}$	$2\theta^\circ$	$d \text{ \AA}$	$2\theta^\circ$	$d \text{ \AA}$	$2\theta^\circ$	$d \text{ \AA}^\circ$
1	14.925	5.9308	14.99	5.9054	14.950	5.9210	-	-	14.601	6.0613
2	-	-	23.590	3.7683	23.535	3.7770	23.585	3.7692	23.795	3.7361
3	-	-	29.720	3.0035	29.750	3.0006	29.665	3.0091	29.034	3.0728
4	30.110	2.9655	30.135	2.9631	30.110	2.9655	30.340	2.9436	30.383	2.9394
5	35.475	2.5284	35.990	2.4934	35.975	2.4944	35.690	2.5137	35.599	2.5198
6	40.980	2.2005	40.815	2.2090	40.810	2.2093	-	-	-	-
7	43.910	2.0602	43.555	2.0744	43.580	2.0751	-	-	43.990	2.0565
8	45.105	2.0084	45.890	1.9759	45.835	1.9781	45.425	1.9950	-	-
9	46.345	1.9575	46.550	1.9494	46.515	1.9507	-	-	46.730	1.9422
10	-	-	48.085	1.8907	48.055	1.8918	48.070	1.8913	-	-
11	50.025	1.8218	49.960	1.8240	49.940	1.8247	-	-	-	-
12	-	-	50.910	1.7922	50.890	1.7928	50.550	1.8041	50.387	1.8095
13	52.535	1.7405	52.665	1.7365	52.690	1.7357	-	-	52.894	1.7295

13 might be associated with oxalate phase in calculi, whereas reflections 2, 3, 10 and 12 might be due to the phosphate phase of calculi. However, the common reflections 4, 5 and 8 in both calculi might be assigned to the presence of calcium atom or content. The other reflections that did not match in XRD patterns might be due to impurities and organic matrix of the calculi. Thus, it could be concluded that calculi were of COM type with certain phosphate impurities. It was also found that calculus II contained all the reflections of struvite crystals, indicating the presence of struvite impurity phase.

The present analysis also suggested that COM phase was present in the calculi. This also correlated with the amount of water molecules found associated with the calculi from the TGA study. The simple powder XRD technique without any special up-gradation was powerful enough to identify the constituents of major phase in the calculi, which is very important for clinicians to decide the therapy or medical management to avoid the recurrence, even though it is not sufficient to identify the amount of various impurity present in the calculi.

Scanning electron microscopy (SEM)

The appearance of urinary calculi by SEM permits identification based on textural grounds³⁵⁻³⁷. Figure 5a shows a part of urinary calculus I having the growth within the matrix with certain empty spaces (Magnification 1.01 kX). Further magnification showed the reticulate-type of appearance and boundaries of small crystallites as shown in Fig. 5b, where individual whewellite (COM) crystals were observed with their clear morphologies

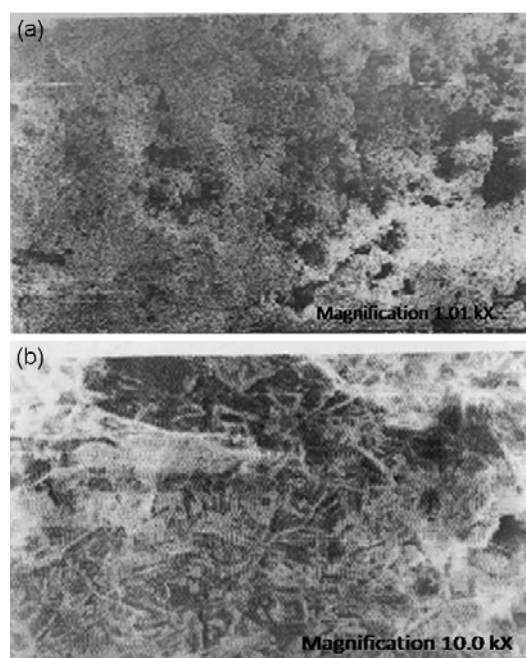


Fig. 5—SEM of calculus I [(a) Showing growth within matrix with empty patches at 1.01 kX; and (b) exhibiting the reticulate type of appearance and boundaries of small rectangular crystallites at 10.0 kX]

(Magnification 10.0 kX). The calculus I in the present study was found to be of Jackstone-type³⁸.

The SEM of calculus II is shown in Fig. 6a (1.08 kX magnification). By applying extra high tension (EHT) of 10 kV, the irregular texture with some empty spaces could be observed. At 5.00 kX magnification (Fig. 6b), the fibrous-type growth features were observed, instead of the reticular crystallite-type morphologies, in contrast to calculus I. This could be due to the growth of phosphate

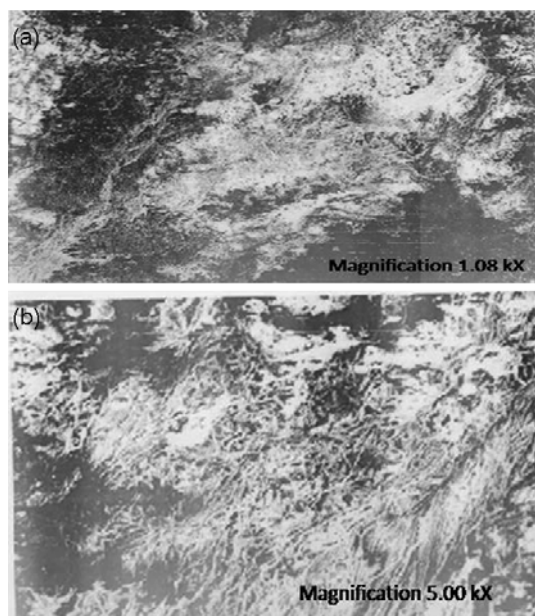


Fig. 6—SEM of calculus II [(a) Showing irregular texture with some empty space at 1.08 kX; and (b) showing the fibrous type growth features at 5.00 kX]

within the whewellite irregular structures. This suggested that structure of calculus II was of a different type from that of calculus I, as also revealed by TGA.

EDAX

The commonly chosen identification methods may often found to be inaccurate, because very rare calculi are pure and most of the calculi are mixed stones containing two or more compounds, many of them presenting typical frequently occurring combination just like mineral parageneses found in rocks. The EDAX technique has been employed to investigate the composition of different types of urinary calculi³⁶. The elemental analysis of urinary calculi is reported by laser-induced plasma spectroscopy (LIPS). The LIPS is expected to allow the real-time clinical measurements of elemental contents and concentrations of biomaterials without any rigorous sample preparations³⁹.

For EDAX analysis, the accelerating potential of 5 kV was applied and the system resolution was of 131 eV. Table 3 shows the results for the calculus I and II. Only a trace of phosphorus was obtained in the calculus I. The EDAX technique provided the exact percentage of calcium, oxygen and phosphorous in calculi. The presence of phosphorous was very minor and indicated the major phase of calcium oxalate.

Table 3—EDAX results for calculus I and II

Element	Apparent conc.	Stat. Sigma	k Ratio	k Ratio Sigma	Atomic %
Calculus I					
O K	203.390	5.188	3.81954	0.09743	47.36
P K	3.788	2.405	0.12314	0.07817	0.39
Ca K	430.283	25.649	12.54468	0.74778	52.25
Calculus II					
O K	31.570	1.438	0.59286	0.02701	43.94
P K	1.067	0.504	0.03468	0.01637	0.65
Ca K	77.575	7.828	2.26166	0.22823	55.40

Conclusion

It was found that various techniques used for analysis of urinary calculi have merits and demerits. FT-IR and powder XRD techniques are quite rapid, comparatively simple for sample preparation and quite capable to identify the major phases present in the particular calculus; however, they cannot help in identifying amorphous phase and water of hydration. Thermal technique is capable to identify the water of hydration in calculus, but does not provide any information on the type of impurity phase present in the major phase of calculi, i. e., phosphate or oxalate type calculi. Microscopic features of the calculus can be studied by SEM, which helps in identifying the arrangement of microcrystalline phase in the matrix of calculi. Thus, no single technique can provide the complete information of the composition and structure of urinary calculi and thus requires another suitable technique for complementary information. However, based on the accuracy of powder X-ray diffraction results and capability to identify the major phase, the clinicians can get adequate information to treat the patient appropriately. The exact estimation of elemental contents is only possible by techniques like EDAX and LIPS.

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