International Journal of Current Science Research
www.drbgrpublications.in

Volume: 2; Issue: 4; April-2016; pp 591-602. ISSN: 2454-5422

AN ANALYTICAL STUDY AND CHARACTERIZATION OF THERMAL AND FTIR STUDIES OF URINARY CALCULI

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Abstract

Urolithiasis is identified to be a major urological disorder affecting people all over the world irrespective of their religion, sex and caste. Urinary stone samples resected from the urinary bladders of two patients belonging to seashore areas of kanyakumari District of Tamil Nadu State, India are investigated by using XRD,SEM, TGA, DSC and FTIR to understand its chemical structure. Calcium phosphate shows exothermic peak around 200.02°C is due to the decomposition with the evolution of CO and cracking of the remaining products. Results of analytical studies reveal that samples under investigation consist mainly in calcium phosphate and calcium oxalate hydrate.

Keywords: Urinary calculi, calcium phosphate, calcium oxalate hydrate, XRD, FTIR, TGA, DSC

Introduction

Urolithiasis is identified to be a major urological disorder affecting people all over the world irrespective of their religion, sex and caste since antiquity (Ali & Nambi Raj 2008; Kalkura 1993]. The increasing incidence of crystal deposition diseases such as urinary, kidney and gall stones in people of all ages affecting a considerable number of the total population is a major social and economic problem, considering the number of days lost from work and cost of

hospitalization (Kalkura *et al.,* 1993]. Growth of urinary calculus usually occurs around an initially formed nucleus, some calculi comprise a single component, the majority has 'mixed' structures (Rodgers *et al.,* 1982). Most of the urinary stone samples compose mineral crystals aggregated into random clumps of varying sizes that are formed within the kidney in a relatively open environment by processes not orchestrated by specialised cellular or macromolecular machinery (Phulwinder and Ryall 1994).These deposits have either any of the constituents like calcium phosphate, cystine, hrdroxyapatite, uric acid, calcium oxalate or a mixture or some combinations of afore said constituents. Apart from this morphology, size and shape also vary from patient to patient which are determined by their food habit as well as living conditions. Several authors reported the composition of these stones based on different analytical techniques. Knowledge of the composition of the stones will help to determine the underlying causes of stone disease, in an attempt to prevent its recurrence, and this had a direct impact on the choice of treatment (Cytron *et al.,* 2003). Thus knowing the exact chemical composition of the urinary calculi is of great importance not only because of its relationship with dietary and other health factors, but also in the prevention of recurrent urolithiasis (Sathish *et al., 2008*).The exact knowledge of chemical composition and its structure will help to design and develop new drugs for the treatment of urinary stones. A few papers has reported the composition analysis of urinay stones resected from patients belonging to tropical region. In the present work urinary stones resected from some patients hailing from kanyakumari District of Tamil Nadu State, India are analysed to understand their chemical composition and structure.

Materials and Methods

The samples used in the present investigation were surgically removed from two patients suffering from urolithiasis. The obtained samples were washed several times in ethanol and distilled water allowed to natural drying for a period of two months in room temperature

(32^{o}C). The XRD patterns of the well powdered samples were collected by using Bruker AXS D8 Advance diffractometer with CuKα radiation having wavelength 1.5406 A^{o}. The TG curves of the samples in the present investigation were taken using a Perkin Elmer, Diamond TG/DTA(STA 6000) with a temperature accuracy of ± 0.20% in the temperature range of 40 to 740^{o}C. Differential scanning calorimetric studies were carried out on a Mettler Toledo system(DSC 822^{e}) and the temperature range from 30-450^{o}C with a temperature accuracy ±0.2^{o}C. The SEM images of the samples were taken by using JOEL MODEL JSM 6390 LV, 20 kV microscopes. FTIR spectra of the samples were recorded by KBr pellet method in the wavelength region 400–4000 cm-1 with Thermo Nicolet, Avatar 370 FTIR spectrometer.

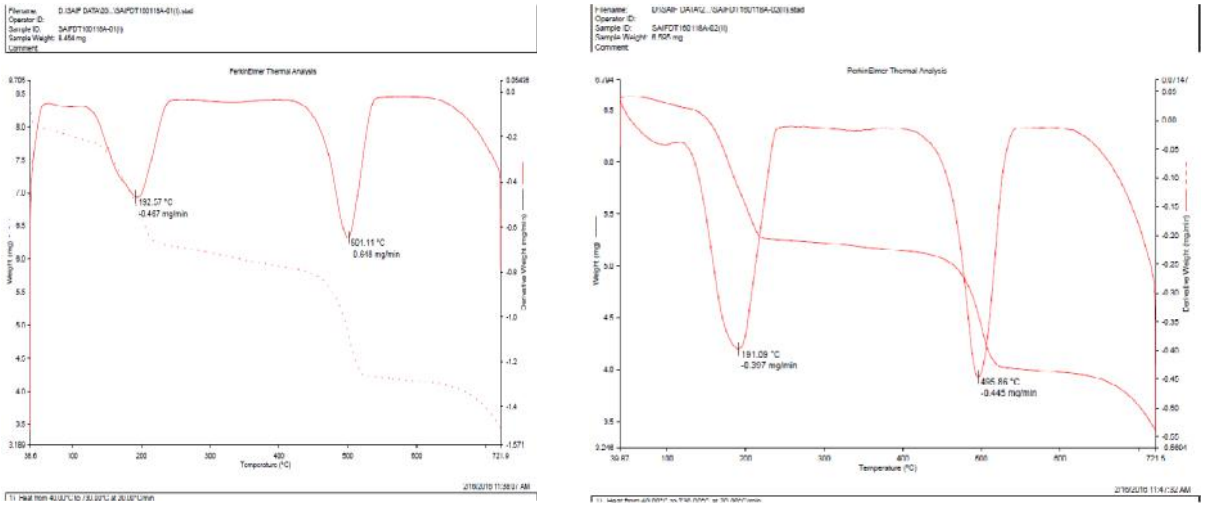

Fig.1. TGA/DTA curve of sample type 1 (calcium phosphate) and sample type 2 (calcium oxalate hydrate)

Results and discussions

Urinay calculi usually contain combinations of inorganic as well as organic constituents with some elemental metals and are highly variable in their composition (Ali & Nambi Raj 2008). XRD patterns of the samples 1 and 2 show well defined peaks indicating crystalline nature of the samples under investigation. The DSC analysis of sample 1 shows strong endothermic peak at 60.86^{o}C with a weak endothermic peak around 200.02^{0}C (Fig.3).

Nevertheless, an endothermic peak is observed at 71.85^{0}C and another peak at 167.87^{o}C with strong one at 810^{o}C. The endothermic peak at 71.85^{0}C corresponds to the loss of the loosely bound water of crystallization from the *calcium oxalate hydrates*. This is confirmed by the observed weight loss from TGA curve of sample 1 at 192.52^{o}C (Rodgers *et al.,* 1982). Later both samples show almost same behaviour. These observations indicate that samples 1 and 2 have slight variations in its composition. An exothermic peak around 200.02°C is due to the decomposition of calcium phosphate with the evolution of CO and cracking of the remaining products in the organic matter in chemical composition of these samples which is in agreement with the decomposition temperature of calcium oxalate hydrate (Sathish *et al., 2008).* Four most intense XRD lines observed in the present samples are matching with those of standard JCPDS patterns (Table 1). Based on this, XRD pattern of Sample 1 can be indexed as *calcium phosphate* (JCPDS-09-0169) while Sample 2 as *calcium oxalate hydrates* (JCPDS-14-0789). This is in agreement with slight variation observed in thermal decomposition behaviour of these samples as described previously. The SEM micrographs show small crystallites of varying size with almost granular nature and some of these are agglomerated (Fig.3).

Table 1. Comparison of d-values of the samples with JCPDS data

Sample 1		**Sample 2**	
d-value (Å)		d-value (Å)	
Observed	**(JCPDS-09-0169) $Ca_3(PO_4)_2$**	**Observed**	**(JCPDS-14-0789) $C_2CaO_4.H_2O$**
3.32276	3.25	3.66574	3.84
3.13110	3.21	3.30386	3.07
2.71118	2.88	2.08	1.99
1.91583	1.980	1.70	1.69

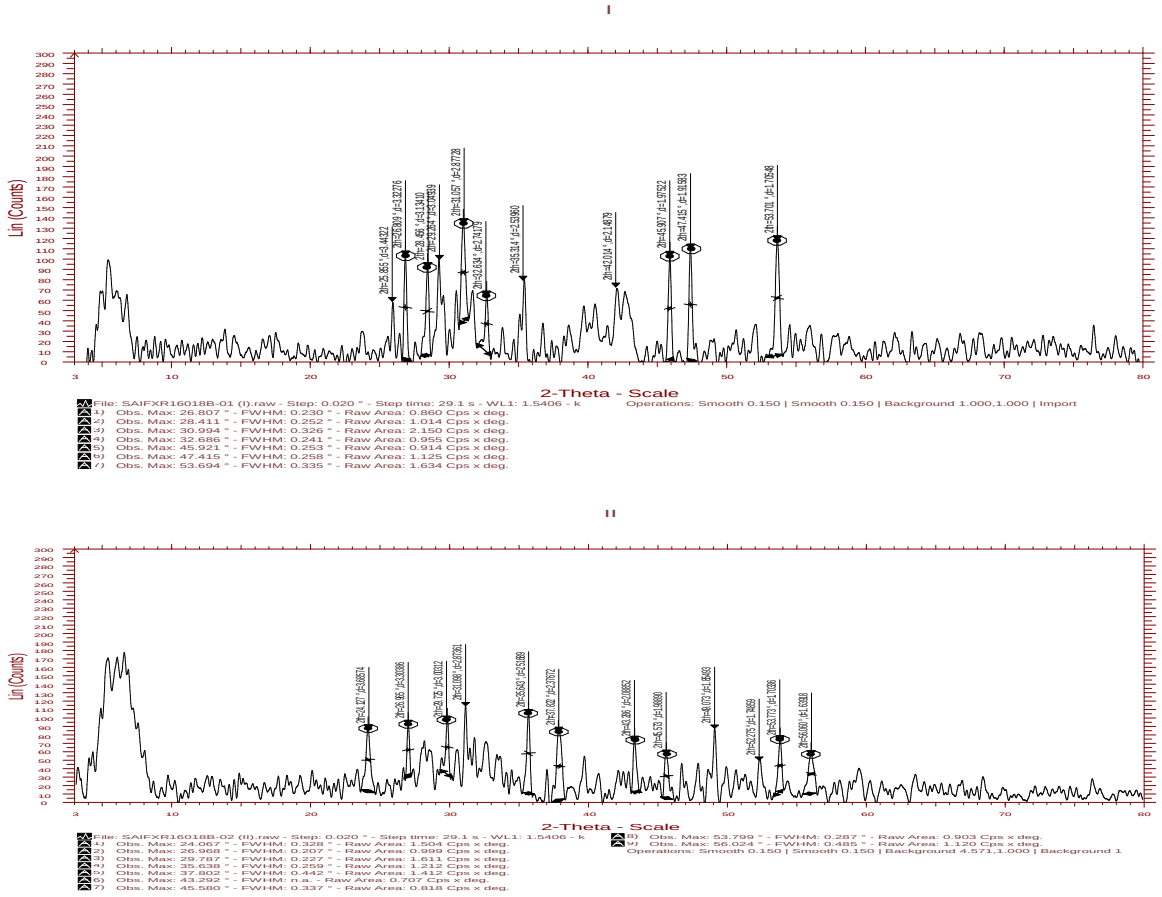

Fig.2. XRD curve of sample type 1 (calcium phosphate) and sample type 2 (calcium oxalate hydrate)

Calcium phosphate is one the major nitrogen-containing excretory products in biological systems and is a naturally occurring antioxidant (Chandra and Huyskens 2007). FTIR spectral patterns of sample 1 & 2 also resemble those reported by Kalkura *et al.*,1993 in uric acid crystals with minor shift in band positions (Table 2.) (Kalkura *et al.*,1993) In sample 1 calcium phosphate is reported at 607.05,780.14,918.96,1031.67,1321.12,1630.94 and 3461.47 cm-1 (Safaa and Mohamad 2007). The most intense band in the IR spectrum of the sample 1 is observed at 1630.94 cm-1 and this is due to C-C stretching vibrations (Wilson *et al.*, 2008). The strong shoulder band at 1630.94 cm-1 indicates the asymmetric deformation of N-H bend and at 1321.12 cm-1 due to the stretching of N-O symmetric stretching (Ali &

Nambi Raj 2008; Banwell and McCash 1995). Bands contributed by OH bending vibrations of H2O molecules are also expected in this region (Bushiri *et al.,* 2008). The C-N stretching vibrations are observed at 1031.67 cm-1 in both the samples (Safaa and Mohamad 2007). The presence of O-H bend deformation vibration at 918.96, N-H wagging at 780.14 cm-1 confirmed the existence of 1^{o},2^{o} amines group (Ramachandran and Natarajan 2004). Further bands due to three *v*CO vibrations which are predicted 514.13 cm−1 in isolated calcium phosphate is not observed in the present Relatively strong absorptions are expected in the IR spectra of the sample 2 due to O-H and N-H stretching vibrations in the frequency range of 2200-3000 cm-1 in an aromatic structure. The band extending from 3200-3500 cm-1 with peaks at 4387.83 cm-1 may be contributed by O-H stretching and H-bonded with alcohols,phenols groups (Safaa and K.Mohamad 2007). The moderately intense IR band at 1621.21 cm-1 is attributed to N-H bendingin 1o amines group(Bushiri et al., 2008). The weak band at 1319.38 cm-1 may be due to the N-O asymmetric stretch of Nitro compounds. The *abinitio* computations predicted four bands corresponds to stretching *v*(NH) vibrations at 951.84,780.15,606.15 and 515.68 for calcium oxalic hydrate (Chandra and Huyskens 2007). These bands may likely to couple each other appears at 3487.83 cm-1 due to solid state effect or due to the presence of metallic traces like calcium. Above analytical results including FTIR investigation indicate that sample 1 is basically calcium phosphate and sample 2 is calcium oxalate hydrate. Reduced urinary pH could be one of the important risk factors for its formation (Ombra et al.,2001). XRD analysis shows the presence of H2O in sample 2, in most chemical environments, the hydroxyl group does not exist in isolation and a high degree of association is experienced as a result of extensive hydrogen bonding with other hydroxyl groups. In its crystalline form calcium oxalic hydrate has a perfect hydrogen bonding system, which makes use of all the hydrogen atoms (Ringertz Acta 1966). Four oxygen atoms and 2 C bonds are available for hydrogen bonding formation in calcium oxalic phosphate structure.

The C bond involved in the interaction with H2O is elongated between values ranging from 0.0107 to 0.0153 Å , this largest elongation corresponds to the smallest NHO intermolecular distance of 1.878 Å. Complex formation also results in an elongation of the OH bond of H_2O by 0.0147 Å again in this case, the largest elongation parallels the shortest OHO intermolecular distance of 1.934 Å. and predicted *v* N9H vibration in this case at 3452 (Chandra and Huyskens 2007). The moderately intense band observed in present sample at 3447 cm-1 which suggest that H2O is coordinated to N9 of calcium oxalic hydratestructure Table 2 (Chandra and Huyskens 2007). Further, the impact of hydrogen bonding is to produce significant band broadening and to lower the mean absorption frequency. Since the symmetric stretching vibrations of O-H occur in the range 2700 up to 3540 cm-1. The broad IR spectral profile in the OH stretching region of sample 2 is attributed to hydrogen bonds (Bushiri et al., 2008). Thus the FTIR spectral signatures are conformity with that of other analytical studies like XRD and thermal analysis used in the present work suggesting that the sample 1 is calcium phosphate and sample 2 is calcium oxalate hydrate.sample and probably it may coupled with ther bands and being shifted to lower frequencies (Chandra and Huyskens 2007).

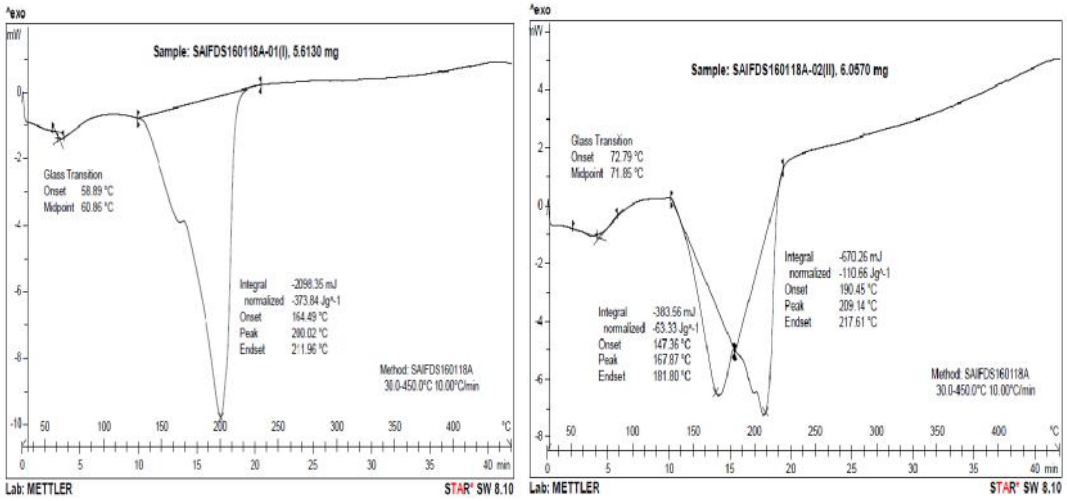

Fig.3. DSC curve of sample type 1 (calcium phosphate) and sample type 2 (calcium oxalate hydrate)

The scanning electron micrographs of the samples under investigation are shown in Fig. 4. The rough and irregular granular type surface morphology is observed from the SEM micrographs of all the samples. But granular size is more in sample 1, and is less in sample 2 and shape of granules are different from that of sample 1. These morphological differences in samples are quite expected since these crystals are having different chemical composition, crystalline structure and growth environment. Phosphate is one of the bases of the DNA and microorganism like E. coli strongly reduce urinary citrate and increase urease-induced calcium phosphate crystallization (Ombra *et al.*,2001). These microorganisms also help the biomineralisation of amorphous calcium carbonate in urinary system (Phoenix and Konhauser 2008). Usually the growth of a urinary calculus occurs around an initially formed nucleus. Some calculi comprise a single component, but the majorities have 'mixed' structure, showing variations in composition from the core to the surface. The large number of possible stone components and the variability in their relative arrangements within a stone add to the structural complexity (Ali & Nambi Raj 2008). Nucleation and the rate of nucleation are important steps in the crystallization process and are also important in determining its size and morphology of the crystals. Nucleation rate, growth and agglomeration depend on the specific conditions like molar concentrations, pH, temperature, etc. in a biological crystallization system (Wilson *et al.,* 2008; Thongboonkerd *et al.,* 2006; Basavaraj *et al.*, 2007). Apart from this different types of trace elements including Mg, Cd, Pb, Zn, Fe, etc., may also affect the crystallization process (Bushiri et al., 2008). There seems to be no doubt that stone formation is predominantly governed by a fixed particle mechanism, in other words, development from crystals into stones occurs in a fixed state (Antonakos *et al., 2007).* Further, the morphology and chemical composition of these crystals depend upon time taken for super saturation and chemical reaction in mediums with varying pH. Once supersaturation condition inside the urinary bladder is achieved, the initial nucleation and formation of initial

seedlings occur as a continuous process. Usually, after urination, the supersaturation condition may cease inside the urinary bladder. In most cases it may take several days for reaching another supersaturation condition inside the urinary bladder system for a favourable crystal growth condition. But, during this time probably, the previously grown crystalline seedlings are covered by a gelatinous mucinous layer (or a thin veil) of macromolecular or cellular (epithelial) material. This layer provides the framework for relatively undisturbed diffusion of the low-molecular-weight components of the crystal phase for further growth. This layer is amorphous in nature and contains different type of proteins and enriched with other carbonated species Banwell and McCash 1995. This carbonaceous component may be in sp3 or sp2 hybridised states or hydrogenated amorphous carbon (a C:H). This is evidenced by weak IR band at 1350cm−1 (a-C) in sample 1 and at 1550cm−1 ((a C:H).) in sample 2 and at 1317 (a-C) cm^{-1} in sample 3 (Banwell and McCash 1995; Antonakos *et al., 2007*). The mucinous layer is continuously incorporated into the growing stone material thus becoming the well known stone matrix (Rey *et al.,* 1991). Supersaturation condition may again occur inside the urinary bladder, and this condition may be different from that of initial one at least several of the parameters like temperature, pH, or mineral concentration may vary. So, the secondary grown or deposits may be slightly varied

in its morphology or shape and thus rule out the formation of defect and inclusion free single crystal. Thus obtained crystal may be an imperfect crystal usually an agglomeration (Fig. 4) of smaller crystallites with different morphology in agreement with the SEM micrographs.

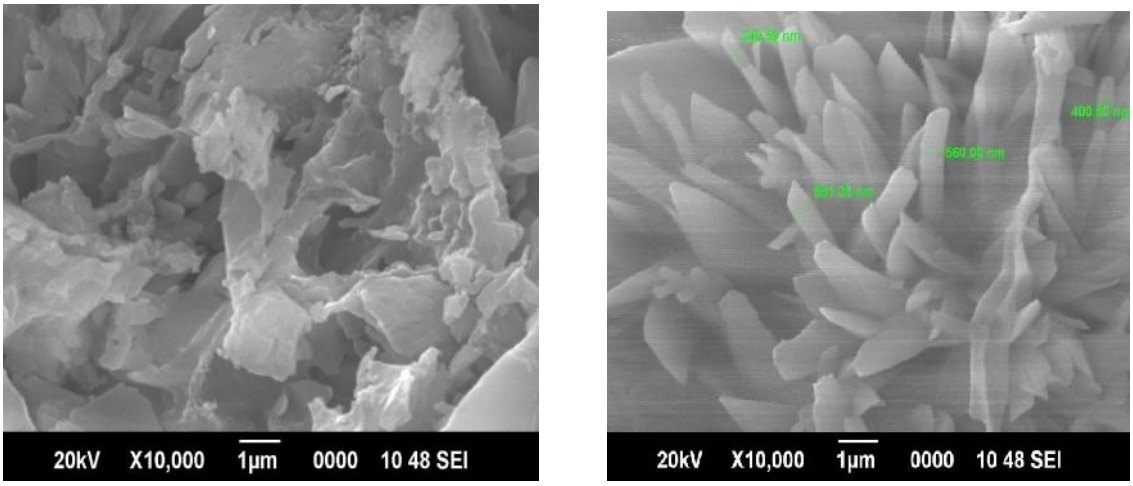

Fig. 4 Scanning electron micrographs of (a) Sample type 1 (calcium phosphate) and sample type 2 (calcium oxalate hydrate)

Table 2. Infrared spectral data (cm-1) samples 1 & 2

Sample 1 Calcium Phosphate **$Ca_3(PO_4)_2$**	Sample 2 Calcium Oxalate Hydrate **$C_2CaO_4.H_2O$**	Assignments
3461.47(s,b)	3487.83(s,b)	O-H stretch, H-bonded
1630.94(m)	1621.21(m)	N-H bend
1321.12(m)	1319.38(m)	N-O Symmetric stretch
1031.67(m)	1034.52(m)	C-N stretch
918.96(m)	951.84(s)	=C-H bend
780.14(s,b)	780.15(s,b)	N-H wag
607.05(m)	606.64(m)	C-Br stretch
514.13(m)	515.68	C-Br stretch

vw - very weak; w - weak; wbr - weak broad; m - medium; vvw - very very weak; sh - shoulder; ms - moderately strong; vvs - very very strong; s –strong; msbr – moderately strong and broad

Conclusions

Urinary stones collected from the urinary bladders of the patients from Kanyakumari District of Tamil Nadu state, India are identified as calcium phosphate and calcium oxalate hydrate. Hydrogen bonding exists in sample 2.

Acknowledgments

The authors are thankful to Dr. Akash, SAIF, STIC, Cochin University for some of the analytical characterisations studies of the present investigation.

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