ANALYTICAL CHARACTERIZATION, THERMAL AND FTIR STUDIES OF URINARY CALCULI

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Urolithiasis is identified to be a major urological disorder affecting people all over the world irrespective of their age, sex and race. Urinary stone samples resected from the urinary bladders of two patients belonging to tropical region, Kollam District of Kerala State, India are investigated by using XRD,SEM, EDAX, TGA, DSC and FTIR to understand its chemical structure. Uric acid shows exothermic peak around 432°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 uric acid and hydrated uric acid. Hydrogen bonding exists in hydrated uric acid samples.

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Keywords: Urinary calculi, uric acid, XRD, FTIR, TGA, DSC

1. Introduction

Urolithiasis is identified to be a major urological disorder affecting people all over the world irrespective of their age, sex and race since antiquity [1, 2]. 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 [2]. Growth of urinary calculus usually occurs around an initially formed nucleus, some calculi comprise a single component, the majority has 'mixed' structures [3]. 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 [4].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 [5]. 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 [6].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 Kollam District of Kerala State, India are analysed to understand their chemical composition and structure.

2. Experimental

The samples used in the present investigation were surgically removed from two patients suffering from urolithiasis. The as obtained samples were washed several times in ethanol and distilled water allowed to natural drying for a period of three months in room temperature (30^0C). The XRD patterns of the well powdered samples were collected by using Philips PW1840 X-ray diffractometer with CuKα radiation having wavelength 1.5406 A^0. The TG curves of the samples in the present investigation were taken using a STAR TGA/SDTA851 with a temperature accuracy of ± 0.25% in the temperature range of 25 to 800^0C. Differential scanning calorimetric studies were carried out on a Mettler Toledo system. The SEM images of the samples were taken by using FEI Sirion FESEM, 30 kV microscope. FTIR spectra of the samples were recorded by KBr pellet method in the wavelength region 400–4000 cm^{-1} with Bruker IFS 66v FTIR spectrometer.

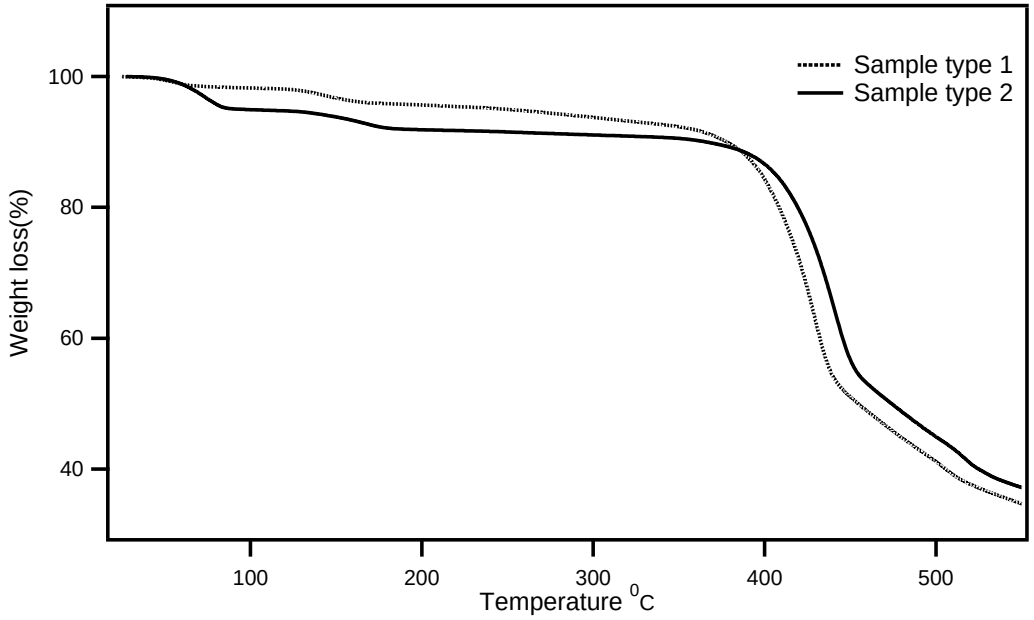

Fig.1. TGA curve of sample type 1 (Uric acid) and sample type 2 (Hydrated uric acid)

3. Results and discussion

Urinay calculi usually contain combinations of inorganic as well as organic constituents with some elemental metals and are highly variable in their composition [1]. XRD patterns of the samples 1 and 2 show well defined peaks indicating crystalline nature of the samples under investigation. The TGA curve of sample 2 shows a slight change in weight loss at 81^0C as compared to that of sample 1 (Fig.1). Later both samples show almost same behaviour. These observations indicate that samples 1 and 2 have slight variations in its composition. The DSC analysis of sample 1 shows strong endothermic peak at 432^0C with a weak endothermic peak around 176^0C (Fig.2). Nevertheless, an endothermic peak is observed at 80^0C and another at 179 with strong one at 436^0C. The endothermic peak at 80^0C corresponds to the loss of the loosely bound water of crystallization from the hydrated uric acid. This is confirmed by the observed weight loss from TGA curve of sample 2 at 81^0C [6]. An exothermic peak around 432°C is due to the decomposition of anhydrous uric acid 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 uric acid [6]. XRD patterns of natural samples are difficult to fit that of JCPDS library. But three 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 uric acid (JCPDS-28-2016), while Sample 2 as uric acid hydrate (JCPDS-19-1996). This is in agreement with slight variation observed in thermal decomposition behaviour of these samples as described previously. EDAX analysis of sample 1 and 2 shows the presence of elements like C, O and N with traces of Ca. 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-28-2016) $C_4(NH)_2O_2C(NH)_2O$**	**Observed**	**(JCPDS-19-1996) $C_5H_4N_4O_3.2H_2O$**
3.10	3.093	3.17	3.20
3.18	3.087	3.12	3.15
4.90	4.904	8.56	8.75

Uric acid is one the major nitrogen-containing excretory products in biological systems and is a naturally occurring antioxidant [7]. It posses bond lengths in its free form, C-C = 1.50, C = C = 1.33, C-N = 1.47, C = N^+ =1.24, C-O^- =1.45, C=O=1.20 A^0 [8]. Specific identifiable peaks in the IR spectrum of uric acid is reported at 710, 750, 780, 1590 and 1675 cm^{-1}[9]. Similarly, Raman spectroscopy is a complementary technique to IR and Kodati *et al* observed Raman bands at 472, 562, 627, 784, 885, 999, 1039, 1122, 1234, 1288, 1046, 1499, 1595, and 1652 cm^{-1} [10,11]. IR spectral patterns of the samples under investigation show similarity with those of aforesaid vibrational bands. This indicates that present samples have uric acid like chemical composition (Table 2.) Further, FTIR spectral patterns of sample 1 & 2 also resemble those reported by Kalkura *et al* in uric acid crystals with minor shift in band positions (Table 2.) [2]. IR bands at 2817, 3018, 1349, 1122 and 877 cm^{-1} in the present samples further confirm the presence of uric acid in the present samples [6]. The most intense band in the IR spectrum of the sample 1 is observed at 1122 cm^{-1} and this is due to C-C stretching vibrations [12]. The strong shoulder band at 1593 cm^{-1} indicates the asymmetric deformation of NH_3 and at 1673 cm^{-1} due to the stretching of carbon-oxygen double bond [1, 13]. Bands contributed by OH bending vibrations of H_2O molecules are also expected in this region [14]. The C=O stretching vibrations are observed at 1349 cm^{-1} in both the samples [9]. The presence of CH2-CO deformation vibration at 1400, CH2 wagging at 1309 and rocking vibrations at 784 cm^{-1} confirmed the existence of CH2 group [15]. Further bands due to three $\nu C{=}O$ vibrations which are predicted between 1772 and 1837 cm^{-1} in isolated uric acid is not observed in the present sample and probably it may coupled with other bands and being shifted to lower frequencies[7].

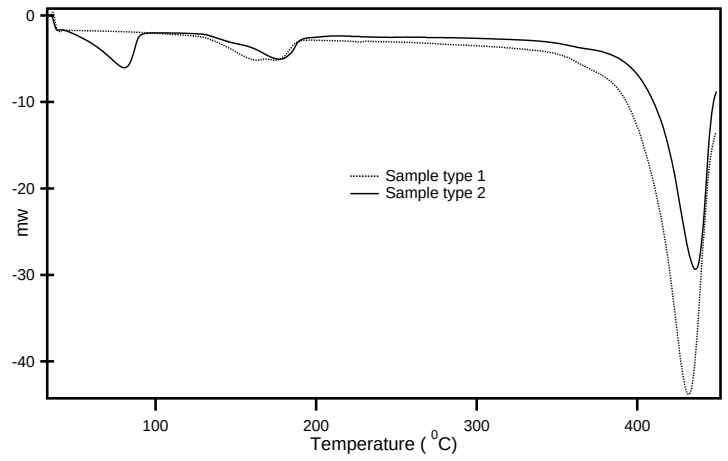

Fig.2. DSC curve of sample type1 (Uric acid) and type 2 (Hydrated uric acid).

Relatively strong absorptions are expected in the IR spectra 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 2100-3300 cm^{-1} with peaks at 2814 and 3010 cm^{-1} may be contributed by O-H and N-H stretching and CH_2 groups [9]. The moderately intense IR band at 1026 cm^{-1} is attributed to C-N stretching [14]. The weak band at 2025 cm^{-1} may be due to the asymmetric stretch of C=C bond. The *abinitio* computations predicted four bands corresponds to stretching *v*(NH) vibrations at 3684, 3672, 3647 and 3611, for uric acid [7]. These bands may likely to couple each other appears at 3853 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 uric acid. Relatively low percentage (3.4%) of patients develops pure uric acid stones [16]. Reduced urinary pH could be one of the important risk factors for its formation [17]. Uric acid stones can also be developed from excessive intake of meat and fish, although hyperuricosuria results from high dietary intake of beef, poultry and fish [18].

XRD analysis shows the presence of H_2O 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 uric acid has a perfect hydrogen bonding system, which makes use of all the hydrogen atoms [8]. Three oxygen atoms and 4 NH bonds are available for hydrogen bonding formation in uric acid structure. The NH bond involved in the interaction with H_2O is elongated between values ranging from 0.0107 to 0.0153 Å , this largest elongation corresponds to the smallest NH**…**O 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 OH**…**O intermolecular distance of 1.934 Å. and predicted *v* N9H vibration in this case at 3452[7]. The moderately intense band observed in present sample at 3447 cm^{-1} which suggest that H_2O is coordinated to N9 of uric acid structure Table 2[7]. 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 [14]. Thus the FTIR spectral signatures are conformity with that of other analytical studies like XRD, EDAX and thermal analysis used in the present work suggesting that the sample 1 is uric acid and sample 2 is hydrated uric acid.

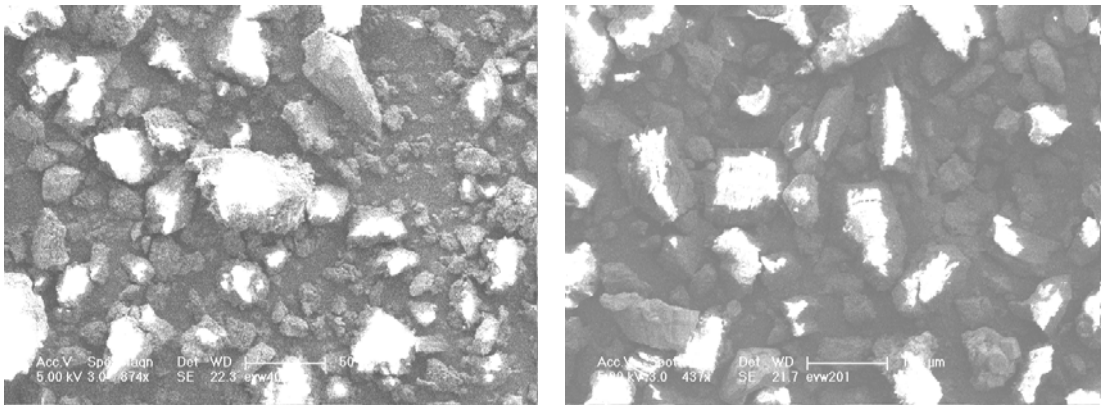

Fig. 3 Scanning electron micrographs of (a) Sample type 1 (Uric acid) and (b) Sample type 2 (Hydrated uric acid).

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

Sample 1: Uric Acid $C_4(NH)_2O_2C(NH)_2O$	Sample 2: Uric Acid Hydrate $C_5H_4N_4O_3.2H_2O$	Assignments
619 ms 655 wsh	619 ms	libr. H_2O
705 s	706 s	libr. H_2O
744 s	745 s	C-C stretch, N-H out-of-plane bending C-N stretching of aromatic
784 s	781 s	Rocking CH_2
877 m	877 ms	C-C stretch, N-H out-of-plane bending
992 s	992 s	C-C stretch
1026 ms	1026 ms	N-H stretching C-N stretch
1122 s	1122 s	C-C stretch
1222 wsh	1222 wsh	ν (CN),
1309 s	1311 s	δCH_2 wagging
1349 s	1349 s	ν C=O
1400 m	1401 m	CH2-CO deformation
1436 m	1436 m	CH_2-CO bending
	1486 sh	δ (CH_2) scissoring
1592 ssh	1593 ssh	asymmetric deformation of NH3
1673 vvs br	1673 vvs br	C=O stretch, ν_2 O-H
2025 w	2025 w	asymmetric stretch of C=C
2817 s	2814 s	ν (CH_3) asymmetric and N-H stretch
3018 s	3010 s	ν_1- ν_3 O-H ν (CH_3) asymmetric
	3447 msbr	*ν* N9H ν_1- ν_3 O-H
3842 wsh	3853 wsh	NH asy. stretch
	3979 wsh	δC-O-H , ν_3 O-H

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

4. Conclusions

Urinary stones resected from the urinary bladders of patients from Kollam District of Kerala State, India are identified as uric acid and hydrated uric acid. Hydrogen bonding exists in sample 2, and H_2O is coordinated to N9 of uric acid structure.

Acknowledgments

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