Solid-State Analysis of Some Urinary Calculi

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Solid-state characterization of urinary calculi using two instrumental, fast and reproducible techniques, namely thermal analysis and FTIR spectroscopy was realised for the investigation of qualitative analysis of nine urinary stones. The applications of these methods in our work were corroborated with already published results for pure compounds: oxalate, phosphate and uric acid. The carried analysis confirmed the presence of five simple stones, consisting in COM, uric acid and struvite, and four mixed stones, consisting in COM+Uric acid and COM+organic traces, respectively. A discussion regarding a correlation between thermal behaviour and FTIR spectrcoscopy was presented. The obtained results concerning the composition of urinary calculi are important for the clinical analysis in the prophilaxy and methaphylaxy of urolithiasis.

Keywords: kidney stone, urinary calculi, oxalate, struvite, thermal analysis; FTIR spectroscopy.

Renourinary lithiasis (RUL) and microlithiasis (RUM) or the formation of kidney stones is known and studied since antiquity, and lithotomy is considered one of the earliest surgical procedures [1]. Nowadays, RUL is considered a medical disorder with a wide distribution and plurifactorial determinism. The multitude of problems which are raised and forms nowadays the complex research involved in the field of interdisciplinary domain – RUL and RUM, appeared and defined over the centuries. The renourinary calculus is the final product obtained as a consequence of numerous and complex physico-chemical and hemodynamic modifications, which were not completely described up to date. Knowing the pathophysiology and the mode of formation of different parageneses that characterize the reno-urinary concretions is an essential issue for the elucidation of complex mechanism of lithogenesis. The importance of knowing the composition of calculi resides on the fact that in the case of disease recurrence, a strategy for the treatment can be obtained [2].

The evaluation of composition for renourinary concretions (calculi) can be realized by several instrumental techniques, such as thermal analysis [3-6], X-ray powder diffraction [7-8], laser ablation methods [9], as well FTIR spectroscopy and thermal analysis [10-11], or complementary techniques such as thermal, UV, FTIR and XRD studies [12]. Several studies reported the use of hyphenated techniques, such as thermogravimetric analysis with electron dispersive spectroscopy [13].

Urinary stones can contain one component (simple calculi) or more (mixed calculi, containing two or more components), both inorganic compounds (such as carbonates and phosphates), but as well organic derivatives (oxalic acid, uric acid, cholesterol, xanthine and cystine).

By far, the most common type of urinary calculi contains calcium, typically as oxalate with/without phosphate, as apatite or brushite [14]. The formation of calcium oxalate calculi is associated with the presence of calcium oxalate crystals in the urine, due to hyperoxaluria [15]. Medical conditions, such as renal tubular acidosis (RTA) [16] or hyperparathyroidism [17] can determine the apparition of urinary calculi that contain calcium phosphate stones.

Uric acid is presented in about 5–10% of urinary calculi [18] especially in the case of metabolic abnormalities and obesity [19]. Uric acid urinary calculi can be associated with hyperuricosuria with/without hyperuricemia, or with dysfunctions of of acid/base metabolism where the urine is highly acidic and uric acid crystallize [20].

Struvite (ammonium magnesium phosphate, $NH_4MgPO_4\cdot 6H\text{-}_2O$) calculi have an incidence under 15% of urinary calculi. Their presence is generally associated with the presence of an urinary system localized infection. In this case, by the use of an enzyme (urease), urea-splitting bacteria metabolize urea into $NH\text{-}_3$ and CO_2. The presence of these metabolites determines the modification of urine *p*H (an increasing of *p*H), determining physical conditions for the formation of struvite stones. The species that determine these infections were previously studied [21].

Several rare metabolic errors from genetic provenience can determine the accumulation of different compounds in urine that can influence the formation of crystals. The formation of cystine calculi can be favorized by patients suffering from Fanconi syndrome, cystinuria or cystinosis. Patients affected by xanthinuria often produce calculi composed of xanthine. Urolithiasis was observed even in the case of using certain therapeutic agents, with crystals of drug forming within the renal tract in some patients currently treated with triamterene [22] indinavir, tazanavir, and more recently darunavir [23].

The tools of solid-state characterization by thermal methods were previously used in our research group for the analysis of pharmaceuticals or potential bioactive compounds [24-31], and as well for different metal complexes [32-35].

In order to extend the field of research for our group, we aimed towards the analysis of nine urinary calculi samples of human provenience, by thermal analysis and FTIR spectroscopy in determining the composition of renal stones, both simple and mixed.

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Experimental part

Materials and methods

Urinary calculi have been collected intraoperative from subjects of different ages hospitalized at the Clinic of Urology (Timiş). All the samples analyses were carried out with subjects agreement. In order to maintain the confidentiality of pacients identities, the samples were labeled as Urinary Stone (US), from US1 up to US9. All the samples were washed with an ethanol–water mixture and then dried at about 40°C for 24h. All the stones were identically processed, namely were milled and sieved, then kept in closed recipients until the analyses were carried out.

The thermal analysis was carried out using a Perkin-Elmer DIAMOND equipment. Samples about 6 mg were heated in aluminium crucibles, up to 550 °C at a heating rate $\beta = 10$ °C·min^{-1}, in synthetic dynamic air atmosphere.

The FTIR spectra were obtained on the Perkin Elmer SPECTRUM 100 spectrometer using the UATR technique on 4000-650 cm^{-1} spectral range, after a number of 24 acquisitions.

Results and discussions

Thermal behaviour

The thermal decomposition of the urinary stones was studied under non-isothermal conditions in air atmosphere. The samples used in the investigation proved to contain uric acid, calcium oxalate monohydrate and ammonium magnesium phosphate hexahydrate. The organic compounds, present in the urinary stones composition, cause the colour of the sample and some exothermal peaks due to the oxidation processes.

TG, DTG and HF curves obtained for the analyzed samples are shown in figures 1-9. It can be seen that the thermal profile of the stones' decomposition is different and quite complex.

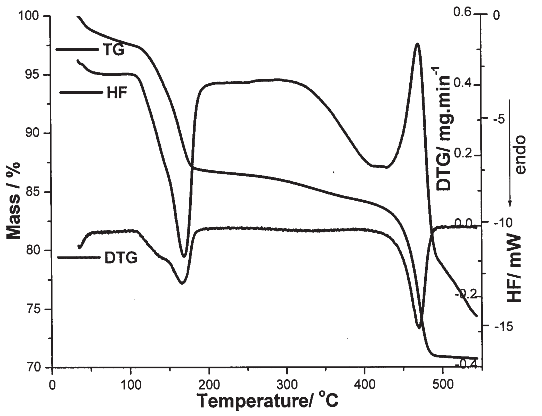

Fig. 1. TG-DTG-HF curves of the powdered sample US1

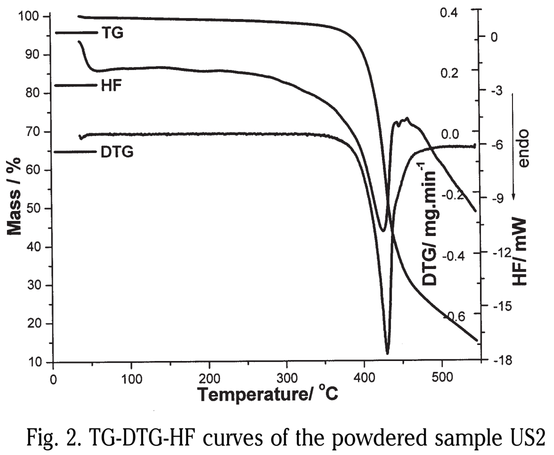

Fig. 2. TG-DTG-HF curves of the powdered sample US2

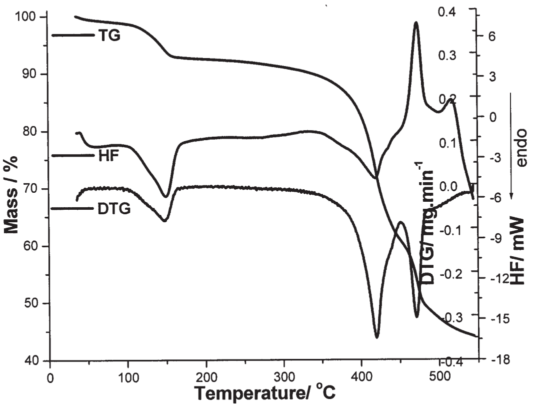

Fig. 3. TG-DTG-HF curves of the powdered sample US3

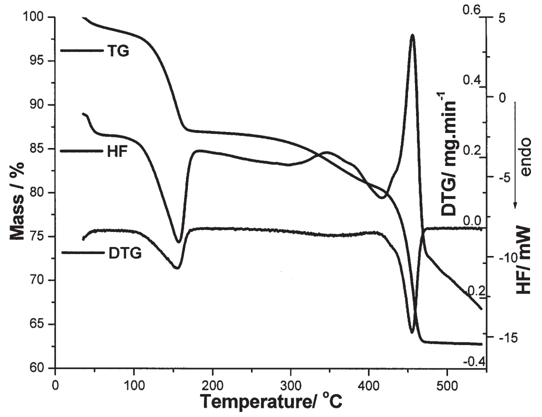

Fig. 4. TG-DTG-HF curves of the powdered sample US4

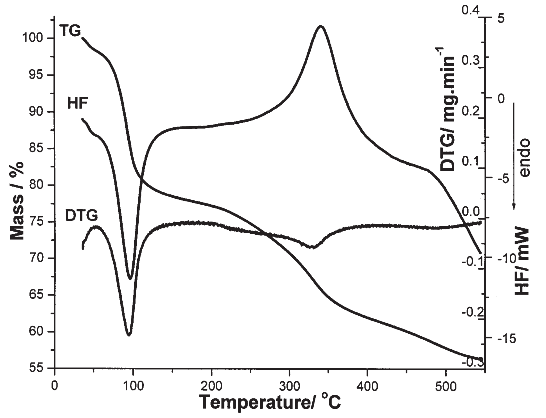

Fig. 5. TG-DTG-HF curves of the powdered sample US5

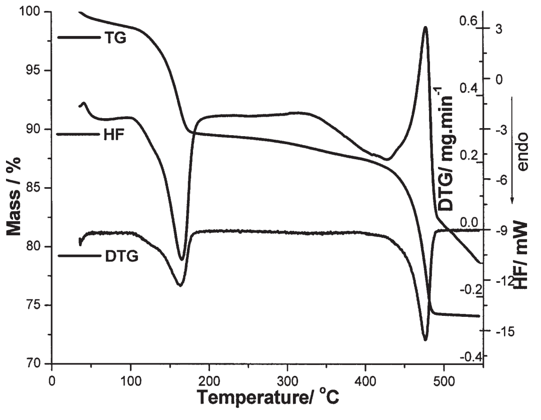

Fig. 6. TG-DTG-HF curves of the powdered sample US6

The thermal analysis results of the nine urinary stones (US1-9), determined in non-isothermal conditions at $\beta = 10$ °C·min^{-1} are summarized in table 1.

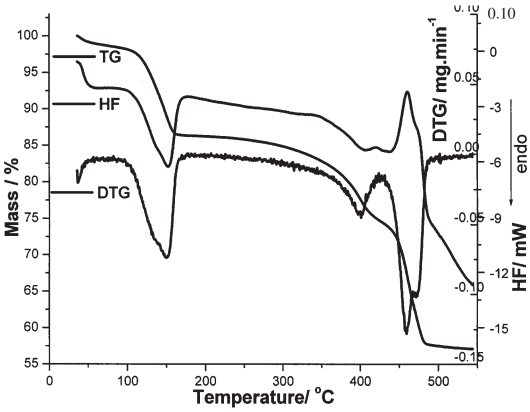

Fig. 7. TG-DTG-HF curves of the powdered sample US7

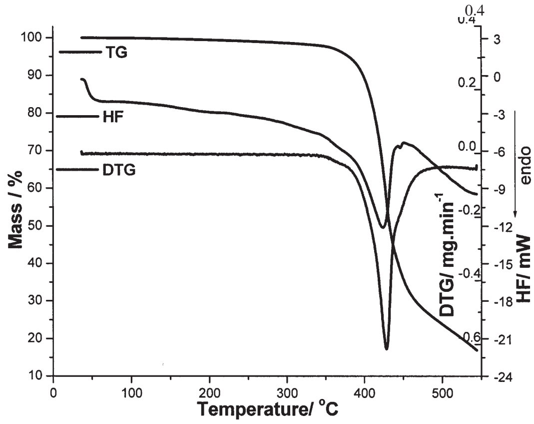

Fig. 8. TG-DTG-HF curves of the powdered sample US8

Calcium oxalate monohydrate (COM) calculi

In the case of samples US1 and US6, the thermal decomposition occurred in two distinct steps in the temperature range 60-190 °C (HF_{max} = 169 °C) and 395-497 °C (HF_{max} = 470 °C) respectively. The mass losses in these two steps are associated with the elimination of water molecule from calcium oxalate monohydrate (first process) and the liberation of the carbon monoxide from the anhydrous formed calcium oxalate.

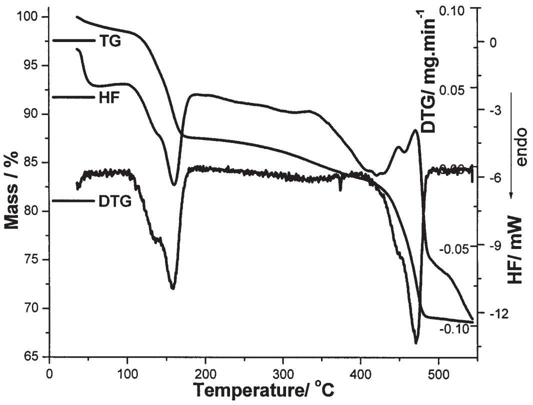

Fig. 9. TG-DTG-HF curves of the powdered sample US9

The thermal profile of US9 sample is very similar to the thermoanalytical curves of US1 and US6, the difference consisting in the presence of organic traces which determined the appearance of new exothermic peak (HF_{max} = 335 °C) due to the oxidation processes of the organic compounds.

Uric acid calculi

The thermal behaviour of US2 is similar with US8, but very different from the other analyzed samples, because these two samples have a different composition: a large amount of uric acid. The single peak observed in the case of these stones (HF_{max} = 425 °C) in the temperature range of 352-505 °C corresponds to the decomposition of purinic structure of the uric acid by giving a strong endothermic effect and a mass loss process which occurs in a single step.

Sample	Process	TG		DTG_{max} (°C)	HF		Thermal effect	Δm (%)
		T_i (°C)	T_f (°C)		$T_{onset\ HF}$ (°C)	$T_{peak\ HF}$ (°C)		
US1	I	53	190	166	106	169	endotherm	13.15
	II	409	495	470	434	469	exotherm	13.08
US2	I	350	505	430	343	425	endotherm	75.87
US3	I	78	166	149	101	149	endotherm	7.23
	II	332	452	420	346	452	endotherm	32.21
	III	452	530	472	458	473	exotherm	15.93
US4	I	66	175	154	99	157	endotherm	12.98
	II	283	415	-	293	348	endotherm	5.65
	III	415	476	454	418	457	exotherm	17.39
US5	I	40	144	94	58	96	endotherm	20.90
	II	220	454	330	254	340	exotherm	19.05
US6	I	88	176	164	102	165	endotherm	10.3
	II	397	497	475	431	478	exotherm	13.18
US7	I	62	169	150	96	152	endotherm	12.49
	II	334	428	401	354	406	endotherm	9.16
	III	428	500	458	438	460	exotherm	16.94
US8	I	352	500	428	341	424	endotherm	81.13
US9	I	78	184	158	98	160	endotherm	11.26
	II	270	406	374	286	335	exotherm	3.41
	III	406	502	471	426	470	exotherm	14.28

Table 1
THERMOANALYTICAL DATA OF THE ANALYSED URINARY STONES

Struvite calculi

TG-DTG-HF curves of US5 sample which contains magnesium and ammonium phosphate hexahydrate, are presented in figure 5. As indicated on the TG curve, the sample starts decomposing at H ≈ 40 °C and the thermal process is relatively completed at H ≈ 550 °C when is obtained the anhydrous form of the double phosphate. The first stage which is a dehydration step continues up to 144 °C and consists in the elimination of three water molecules. In the second stage, 220-454°C, the rest of the water molecules are lost leading to the formation of anhydrous magnesium ammonium phosphate. This second process is overlapping with the oxidation process of the organic traces presented in the analysed urinary stone.

Mixed calculi

The samples US3, US4 and US7 contain a mixture between calcium oxalate monohydrate and uric acid, the difference being the proportion of the two major components. All the three samples present three decomposition steps, accompanied by a mass loss which depends on the composition of the analysed samples. The first two steps between 65-170 °C and 280-450 °C respectively are related to the endothermic elimination of one molecule of water and to the decomposition of the uric acid, respectively. The exothermic peak from the temperature range of 415-500 °C ($HF_{max}H \approx 460$ °C) is associated with the decomposition of anhydrous calcium oxalate with carbon monoxide elimination.

FTIR analysis

The FTIR spectra recorded for all US samples are presented in figure 10 (for samples US1-US5) and figure 11 (for samples US6-US9), respectively.

Calcium oxalate monohydrate (COM) calculi

Literature [12] mentions that COM presents FTIR peaks around 1618 and 1318 cm^{-1} and can be associated mainly with asymmetric carbonyl stretching bands. The FTIR peaks that appear at 1641, 1324, and 1630 cm^{-1} are due to the stretching frequency of metal-carboxylate band. Other reference [6] mentions that FTIR spectroscopy can distinguish between the presence of calcium oxalate monohydrate (COM) and dehydrate (COD), mainly by the analysis of spectral region 960-850 cm^{-1}. COM presents two weak absorptions bands with maximum positions at 952 and 880 cm^{-1}, respectively while COD presents only a singular absorption maximum at 915 cm^{-1}. For COD, the FTIR absorption band around 1630 cm^{-1} in medium in intensity, but broad, while for COM is intense and sharp. Another characteristic band is the one at 780 cm^{-1}, which is broad and less intense in the case of COD, comparative to COM [6].

According to these observations, and corroborating the data with the ones obtained by thermal analysis, we can suggest that urinary calculi samples US1 and US6 are simple samples and consist only from COM, while samples US3, US4, US7 and US9 contain COM along with other components, due to the fact that other bands appear in the spectrum of calculi (table 2). The presence of organic traces in the US9 is suggested by the thermal decomposition profile of the sample, while the presence in traces is suggested by the aspect of the FTIR spectrum, which does not reveal supplementary bands.

Uric acid calculi

Anhydrous uric acid can be identified in the presence of dihydrate from by the analysis of the FTIR spectra [6]. In the case of anhydrous uric acid, the main difference from hydrated form consist in the presence of FTIR peaks in the 1450-700 cm^{-1} spectral domain, associated with the lack of the broad band around 3500 cm^{-1}, which is due to the presence of water in the dihydrate. The anhydrous form of uric acid present two sharp peaks at 1349 and 1309 cm^{-1}, while the dihydrate shows only one relatively broad absorption at about 1330 cm^{-1}. The anhydrous uric acid shows a medium strong peak at 991 cm^{-1} as a weak shoulder in dehydrate [6]. According to these observations, and corroborating the literature data with the ones obtained by FTIR spectra analysis, we can notice that several renal calculi (samples US2 and US8) consist solely in anhydrous uric acid. The presence of uric acid is also noticed in some binary calculi (samples US3, US4 and US7), with different mixing ratio. By corroborating the results with thermal analysis, we can affirm that two samples contain less uric acid than COM (samples US4 and US7), while one sample contain more uric acid than COM (US3) (table 2).

Table 2

ANALYSIS REGARDING THE QUALITATIVE COMPOSITION OF ANALYSED US

Sample	Type	Composition	Observations
US1	Simple	COM	
US2	Simple	Uric acid	
US3	Mixed	COM+Uric acid	Uric acid>>COM
US4	Mixed	COM+Uric acid	Uric acid<<COM
US5	Simple	Struvite	
US6	Simple	COM	
US7	Mixed	COM+Uric acid	Uric acid<COM
US8	Simple	Uric acid	
US9	Mixed	COM+organic	COM>>organic traces

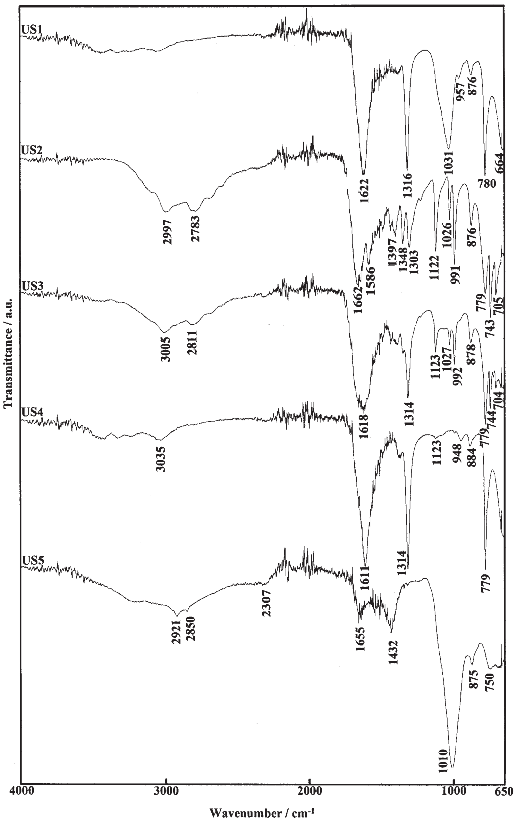

Fig. 10. FTIR spectra for urinary calculi samples US1-US5

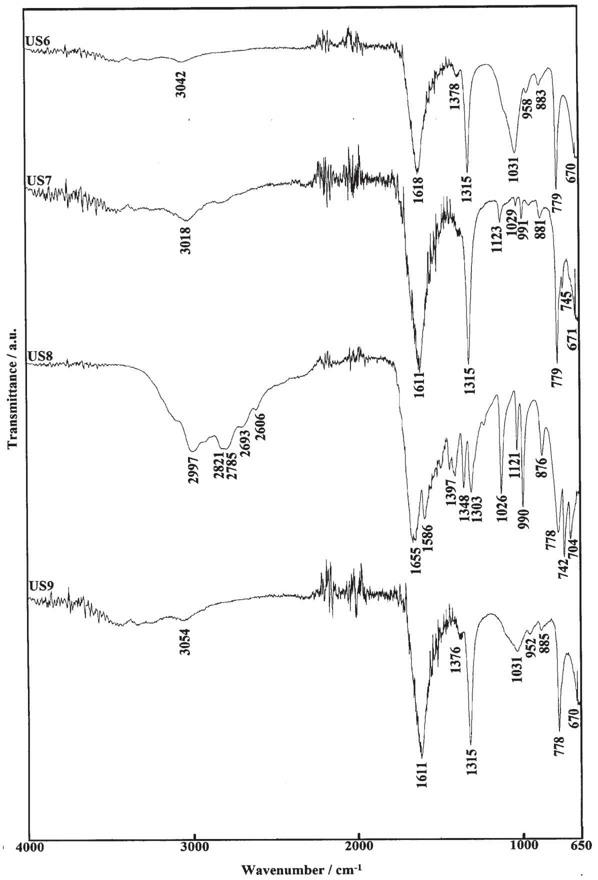

Fig. 11. FTIR spectra for urinary calculi samples US6-US9

Struvite calculi

Struvite shows a broad absorption band between 3300-2500 cm^{-1} and a shoulder at 2304 cm^{-1} due to N-H stretching and bending respectively. A very intense absorption band at 1010 cm^{-1} characterizes phosphate ion. Strong absorption at 1432 cm^{-1} due to NH_4^+ deformation vibration and absorptions noted at 1655, 875 and 750 cm^{-1} are characteristic of struvite calculus US5 (fig. 10, 11).

Conclusions

The composition of nine urinary stones obtained through intraoperative procedure was determined by the use of two instrumental techniques, namely thermal analysis (TG/DTG/HF) and FTIR spectroscopy. The carried analysis confirmed the presence of five simple stones, consisting in COM (two stones), uric acid (two stones) and struvite (one stone), and four mixed stones, consisting in COM+Uric acid (three stones), and COM+organic traces (one stone), respectively. FTIR spectroscopy results are in good agreement with those of thermal analysis.

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Manuscript received: 19.12.2013