

## THERMAL ANALYSIS OF RENAL STONES

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The chemical composition of 200 renal stones, collected from Taxila, Rawalpindi and Islamabad regions in Pakistan, was determined by thermogravimetric (TG) and differential thermal analysis (DTA) techniques. The thermal curves show weight losses at various temperatures indicating dehydration and decomposition phenomena of renal stones. Results were compared with qualitative data obtained by IR analysis which confirmed the chemical composition of various stones in the solid state. The thermal curves helped in the differentiation of various kinds of water held by stones and their chemical composition was obtained by weight loss during pyrolysis. It was found that 26.5% of the stones were pure whewellite, 3% weddellite, 13% uric acid anhydrous, 7.5% struvite, 2.5% ammonium acid urate, 0.5% cystine and 47% stones had mixed composition. In the mixed state the most frequent combinations were those of calcium oxalate with uric acid (14.5%) and with phosphates (27.5%).

**Keywords:** DTA, IR, renal stones, TG

### Introduction

The problem of Urolithiasis is one of the oldest diseases known to afflict mankind. The disease is not a simple one because of the fact that calculi of diverse chemical composition occur in kidneys, ureters and urinary bladders. Urinary stones occur in a wide range of shapes, sizes, colours, compositions and textures.

The importance of knowledge of the chemical composition of renal calculi is well established. The study of the exact chemical composition of urinary calculi is fundamental for the understanding of their etiology, management and prevention of recurrence. A stone can form only when urine is supersaturated with respect to its constituent crystals. The urine of most normal people is supersaturated with respect to calcium oxalate, so, in principle, such stones can form in all people. But in actual fact this does not happen. Some people get stones, while

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others do not. This diverse behaviour must involve some other factors such as diet, environment, genetics etc. Normal urine is not supersaturated with respect to uric acid, cystine, or struvite. Low urine *pH* from hereditary cause or bowel disease promotes uric acid stones, high *pH* from alkali, drugs or renal tubular acidosis increases calcium phosphate supersaturation. Struvite supersaturation occurs only when urine is infected with micro-organisms that possess urease; these are rightly called infection stones. Cystine stones occur only in cystinuria, a hereditary disorder of amino acid transport [1].

The wet chemical methods [2, 3] have been employed for the qualitative analysis of renal calculi since ages, their results were, however, inconclusive in resolving the complex nature of mixed stones. We have taken IR spectra of both pure and mixed stones. IR analysis gives qualitative results and this single technique is not sufficient to get the exact chemical composition of hydrated and mixed stones. Hence attention was paid towards thermal methods of analysis in order to understand the behaviour of various components present in mixed stones and distinguish between anhydrous and hydrated stones in the solid state. In recent years thermal analysis technique is being widely used in the various fields of medical science. The first and most elaborated example in this subject was the thermal analysis of nephroliths [4]. In the present work, TG/DTA method, in addition to the routine IR analysis [5], has been used to understand more clearly the composition of stones in the solid state.

## Experimental

Two hundred renal calculi were collected from the urology unit of the Christian Hospital Taxila and the urology unit of Pakistan Institute of Medical Sciences (PIMS) for a period of three years between April 1986 and June 1989, as the patients presented themselves for operation or they produced the stones for analysis when the stones passed out naturally through urine.

### *Pretreatment*

On reception in the laboratory each stone specimen was documented, its shape and colour noted. In the case of very large stones, an apparently representative portion was selected for analysis. Each stone was cleaned externally with a scalpel and washed free of blood, mucus and other extraneous matter. It was then dried at 50°C in a vacuum and weighed. Each calculus was cut into two nearly equal parts with a small sharp knife. One half of the stone was reduced to a fine powder in an agate mortar. Whereas the nucleus and layers of the other half were carefully singled out and ground separately. The powder was dried again in a vacuum and cooled in a desiccator. Extremely small calculi were taken as such for analysis.

### *Equipments*

During present study, analysis of stones was done by means of

- a) The Shimadzu Thermal Analyser TGA-31, DTA-30.
- b) The Hitachi Infrared Spectrophotometer Model-460.

### *Method for TG*

100–200 mg of powdered sample was put into a ceramic crucible which was suspended in a reaction tube capable of controlling the atmosphere of gases inside the system. Heat was applied at ramping rate of 10 deg/min and thermal diagrams with TG curves from room temperature to 1000°C were obtained. Thermal analysis of all samples of renal calculi was done in inert atmosphere using N<sub>2</sub> as purge gas and with chart speed of 2.5 mm/min [6].

### *Method for DTA*

A powdered sample and reference substance (inert Al<sub>2</sub>O<sub>3</sub>) were put in two separate platinum cells and placed on the dumbbell type detector. Then the sample and the reference substance were heated together at a ramping rate of 10 deg/min.

On the remaining powder of each sample, duplicate determinations of main constituents were performed for comparison first by infrared spectrophotometry [7, 8] and subsequently by established chemical methods of analysis.

## **Results and discussion**

The thermal diagrams of stones taken by both thermal techniques (DTA, TG) were compared with those of pure compounds either commercially available or prepared in the laboratory according to procedures given by Peter Haux [9] as well as reported by Duval [10], Strates [11], Philipsborn [12], Berényi and Liptay [13] and Ainul H. Khan [14]. The comparison helped in the identification of endothermic and exothermic peaks where dehydration, decomposition or loss of the sample occurred. Identification of only those compounds is reported here, which were analysed during the present investigation. The chemical composition, colour and DTA results of representative samples are shown in Table 1. IR absorption bands obtained with renal stones of various compositions are given in Table 2. The number and percentage occurrence of the stone analysed are shown in Table 3.

**Table 1** Differential thermal analysis of renal stones

Chemical composition of stones	Colour of stones	Temperature of reaction / °C			
		endothermic		exothermic	
		range	peak	range	peak
Calcium oxalate monohydrate (pure)		140–250	230	470–535	510
Calcium oxalate monohydrate	Blackish brown	690–805	775	360–405	380
Calcium oxalate mono/dihydrate	Earthy brown	180–290	245	420–470	450
		50–270	230	450–490	470
		680–800	765		
Uric acid (pure)		370–480	450	500–550	510
Uric acid anhydrous	Earthy yellow	370–405	390	490–530	500
Uric acid hydrated	Brownish yellow	410–470	450		
		70–140	120	490–620	550
		375–470	450		
Ammonium hydrogen urate (pure)		40–160	110	500–550	520
		210–260	250		
		275–350	315		
		370–480	450		
Struvite	White	80–250	160	650–700	680
Ammonium hydrogen urate (calculus)	Light brown	200–250	230	480–585	510
		280–370	320		
		390–480	450		
Uric acid and whewellite	Earthy yellow	90–150	130	370–420	410
		160–220	200	450–480	470
		420–450	440	480–520	515
		660–740	720	520–545	530
Whewellite, weddellite and apatite	Earthy brown	150–220	200	310–370	345
		375–420	405	430–480	455
		600–750	710		
Cystine	Brown		165	70–115	100
		170–250	220	470–500	485
		390–465	460	520–590	540
		500–515	505		

Table 1 Continued

Chemical composition of stones	Colour of stones	Temperature of reaction / °C			
		endothermic		exothermic	
		range	peak	range	peak
Whewellite and tricalcium phosphate	Brown	180–290	250	60–155	115
			380		440–490
		670–815	420		
			790		
Struvite and whewellite	Dirty white	90–230	170	350–410	380
		170–260	240	430–480	465
		680–800	770	650–750	680

### Calcium oxalate

The thermal analytical curves of pure whewellite and renal calculus were compared (Table 1). The thermograms of calcium oxalate calculi reveal that bound water in stones is lost in three stages: the mechanically bound water is lost around room temperature to approximately 80°C loosely bound water of crystallisation of the dihydrate to yield monohydrate is lost in the temperature range of 90°–160°C while the water of crystallization of monohydrate in the crystal lattice is discharged at relatively higher temperature around 170°–300°C. The stones always contain non crystalline organic material (matrix) enclosed in an inorganic framework which shows an exothermal decomposition peak at 390°C (Table 1). Decomposition of anhydrous calcium oxalate with the evolution of CO exhibits one exothermic peak at 470°C which is not only due to the removal of CO but also due to the cracking products remaining from the organic matter. The last endothermic dip at higher temperature around 755°C corresponds to the liberation of CO<sub>2</sub> and transformation of CaCO<sub>3</sub> to CaO. The amount of the constant weight residue exceeds that of theoretical amount of CaO, because CaC<sub>2</sub>O<sub>4</sub> calculi frequently contain some phosphates [4].

The two endotherms and one exotherm at about 230°, 755° and 470°C respectively were present in 53 samples indicating the presence of whewellite (calcium oxalate monohydrate, CaC<sub>2</sub>O<sub>4</sub>·1H<sub>2</sub>O). Six samples contained a small endotherm at about 115°C which indicates the presence of weddellite (calcium oxalate dihydrate, CaC<sub>2</sub>O<sub>4</sub>·2H<sub>2</sub>O). From the TG curves the quantities of the two forms of hydrates of calcium oxalate present in the calculi were determined. Using ordinary chemical analysis in the solution form, the composition of the solid is hard to determine. However, using IR spectroscopy composition of solids may be determined to a limited extent. It is known that as the conditions of formation and prognosis of the two kinds of calcium oxalate are different, this determination is of special significance in urological practice [4].

**Table 2** IR absorption bands of constituents of renal stones at various wavenumbers

Chemical composition of stones	Absorption		*Intensity
	frequencies / $\text{cm}^{-1}$	wavelength / $\mu$	
Water	3442	2.91	V.I.B.
	2080	4.81	M.I.B.
	1625	6.15	M.I.S.
	657	15.22	W.
Calcium oxalate monohydrate	3508-3332	2.85-3.00	V.I.B.
	1662-1612	6.02-6.20	V.I.B.
	1323-1315	7.56-7.61	I.S.
	954-945	10.48-10.58	W.
	888-880	11.26-11.36	W.
	784-780	12.76-12.82	I.S.
	666-650	15.02-15.39	M.I.S.
	518-515	19.31-19.42	M.I.S.
424-417	23.59-23.98	V.W.	
Carbonate-apatite	3600-3200	2.78-3.13	W.
	2480-2410	4.03-4.15	W.
	1480-1408	6.76-7.10	I.B.
	1100-1000	9.09-10.0	V.I.B.
	877-870	11.40-11.49	M.I.
	720-710	13.89-14.08	M.I.S.
	606-600	16.50-16.67	W.
	575-570	17.39-17.54	M.I.S.
Hydroxy-apatite	3450	2.90	S.
	1670-1630	5.99-6.14	B.
	1100-1000	9.09-10.0	V.I.B.
	575-570	17.39-17.54	M.I.S.
	606-600	16.50-16.67	M.I.S.
Calcium phosphate	1100-1000	9.09-10.0	V.I.B.
	554-550	18.05-18.18	M.I.S.
	602-600	16.61-16.67	M.I.S.

Table 2 Continued

Chemical composition of stones	Absorption		*Intensity
	frequencies / $\text{cm}^{-1}$	wavelength / $\mu$	
Uric acid	3040–2850	3.29–3.51	Sh.
	1680–1668	5.96–6.00	I.B.
	1596–1589	6.27–6.29	I.S.
	1435–1430	6.97–6.99	M.I.B.
	1403–1398	7.13–7.15	M.I.B.
	1353–1347	7.39–7.42	I.
	1308–1311	7.65–7.63	M.I.
	1126–1122	8.88–8.91	M.I.
	1028–1026	9.73–9.75	M.I.
	995–991	10.05–10.09	M.I.
	880–876	11.36–11.42	M.I.
	783–786	12.77–12.72	M.I.
	747–744	13.39–13.44	M.I.
	704–708	14.21–14.12	M.I.
	620–610	16.13–16.39	M.I.
	576–574	17.36–17.42	M.I.
525–510	19.05–19.61	M.I.	
477–470	20.96–21.28	M.I.	
Ammonium hydrogen urate	3080–2992	3.25–3.34	V.I.B.
	1690–1630	5.92–6.14	V.I.B.
	1436–1427	6.96–7.00	M.I.
	1395–1383	7.17–7.23	M.I.
	1348–1339	7.42–7.47	M.I.
	1275–1268	7.84–7.89	M.I.
	1140–1131	8.77–8.84	M.I.
	1005–1002	9.95–9.98	M.I.
	880–884	11.36–11.31	M.I.
	795–768	12.58–13.02	M.I.
	610–590	16.39–16.95	M.I.
530–520	18.87–19.23	M.I.	
480–490	20.83–20.41	M.I.	

\*M=Medium, I=Intense, V=Very, S=Sharp, Sh=Shoulder, W=Weak, B=Broad

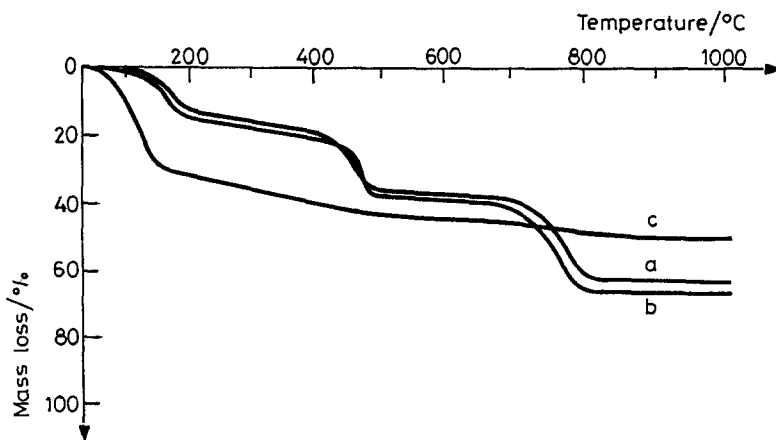
**Table 3** Thermal analysis of 200 renal stones.

Chemical composition	No	%
Whewellite	53	26.5
Weddellite	6	3.0
Uric acid anhydrous	26	13.0
Struvite	15	7.5
Urate	5	2.5
Cystine	1	0.5
Ca-oxalate/Uric acid	29	14.5
Ca-oxalate/Phosphate	55	27.5
Ca-oxalate/Urate	7	3.5
Uric acid/Urate	3	1.5

Number of pure stones=106 (53%)

Number of mixed stones=94 (47%)

TG results show a similar behaviour as those of DTA. Figure 1(a) shows a TG curve obtained for a stone containing whewellite. The first small weight loss of about 0.5% is due to the loss of loosely bound water. The major weight loss occurring at about 150°–250°C is due to loss of water of crystallization. From TG data, the degree of hydration of whewellite was calculated. It has been observed that about 0.8 to 1.5% weight loss at 300°–400°C is due to the destruction of organic matter entrapped in an inorganic framework. The sharp weight change at about 450°C is due to the loss of CO which converts  $\text{CaC}_2\text{O}_4$  to more thermally stable  $\text{CaCO}_3$ . Finally a small weight loss at nearly 700°–800°C is due to the



**Fig. 1.** TG curves of (a) Ca-oxalate monohydrate (b) Ca-oxalate dihydrate (c) Struvite



liberation of  $\text{CO}_2$  converting  $\text{CaCO}_3$  to  $\text{CaO}$ . Calcium oxalate dihydrate (weddelite) stone was identified by TG curve given in Fig. 1(b). About 20% weight loss in the first step indicates the presence of dihydrate. The ratio of the weight loss of two molecules of water (20%), carbon monoxide (17%), carbon dioxide (26.8%) and calcium oxide left as residue (34%) with respect to calcium oxalate, shows the presence of calcium oxalate dihydrate.

Data for IR study of renal stones is given in Table 2. These results show that distinction between calcium oxalate mono- and dihydrate is accomplished by identifying the missing or some additional peaks below  $960\text{ cm}^{-1}$ . The monohydrate exhibits two weak absorptions at  $952$  and  $880\text{ cm}^{-1}$ , while dihydrate instead of these peaks shows only one absorption at  $915\text{ cm}^{-1}$ . In addition the monohydrate shows absorption at  $660$  and around  $515\text{ cm}^{-1}$ , while the former absorption is absent in the dihydrate and in its place there is a broad absorption in the region of  $605\text{--}600\text{ cm}^{-1}$ . The absorption around  $1630\text{ cm}^{-1}$  in the dihydrate is broad and relatively strong. In case of dihydrate the absorption at  $780\text{ cm}^{-1}$  is broad and less intense than in case of monohydrate.

### Uric acid

In Table 1, DTA results of pure uric acid anhydrous show one sharp endothermic peak at  $450^\circ\text{C}$ , while calculi show a sharp endotherm at  $460^\circ\text{C}$  along with other exotherms at  $675^\circ\text{C}$  and a small endotherm at  $495^\circ\text{C}$ . Such a variation may be the effect of various impurities. Very small endotherms at  $120^\circ\text{C}$  is due to the liberation of water indicating the presence of hydrated uric acid. Uric acid calculi form in acidic urine, and are quite soluble in the upper ranges of urinary  $\text{pH}$ . It is

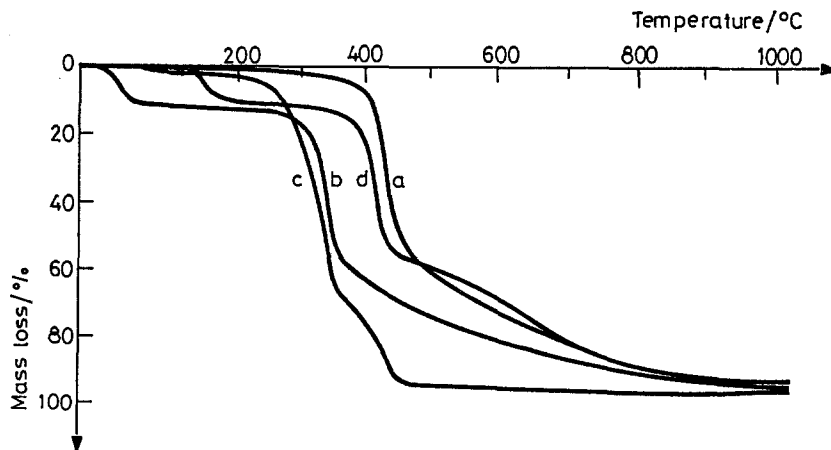


Fig. 2 TG curves of (a) Uric acid anhydrous (b) Uric acid hydrated (c) Ammonium acid urate (d) Cystine

difficult to differentiate by wet chemical analysis between anhydrous and hydrated uric acid and its sodium or ammonium salts by wet chemical analysis. However thermal curves show the difference easily.

A weight loss of about 2% is shown in TG curve (Fig. 2a) for a stone of uric acid anhydrous. The weight loss starts at above 100°C and goes upto 330°C. However the main weight loss is at about 450°C, although there is some tailing off which continues until about 700°C. Figure 2(b) shows the TG curve of stone having the composition of hydrated uric acid. The weight loss at about 100°C was due to water of crystallization. Whereas a major weight loss occurs in the temperature range of 360–450°C.

IR study indicates that anhydrous uric acid can be distinguished from dihydrate by the presence of peaks between the 1450–700  $\text{cm}^{-1}$  region and absence of an extra broad absorption at about 3500  $\text{cm}^{-1}$ , which is due to the water of crystallization in the dihydrate. The anhydrous uric acid exhibits two sharp peaks at 1349 and 1309  $\text{cm}^{-1}$ , while the dihydrate shows only one relatively broad absorption at about 1330  $\text{cm}^{-1}$ . The anhydrous uric acid shows a medium strong peak at 991  $\text{cm}^{-1}$  as a weak shoulder in dihydrate. Similarly the sharpness of a peak at 3000  $\text{cm}^{-1}$  differentiates anhydrous form from the hydrated one.

#### *Ammonium acid urate*

The DTA peak positions of pure ammonium acid urate show three endotherms at 250°, 315°, 450°C and two exotherms at 100°C and 520°C. We noticed a sharp and strong endotherm at 450°C in this case. The result of thermal diagram for a representative ammonium hydrogen urate stone is given in Table 1 which shows that there are two distinct and one somewhat less sharp endotherms at 320°, 450° and 240°C, and one exothermic peak at 515°C. The first pair of endotherms on the DTA curve at 240° and 320°C correspond to the water of crystallization. The next endothermic peak at 450°C corresponds to the decomposition of the sample with the evolution of ammonia. The final exotherm at 515°C also describes the stage of decomposition during which CO is lost. Figure 2(c) shows a TG curve for ammonium acid urate indicating the same behaviour as DTA curve reveals.

The distinction between uric acid and ammonium acid urate has been made by comparing IR spectra of both compounds especially in 625–400  $\text{cm}^{-1}$  region, in which the uric acid has characteristic sequence of four bands but ammonium acid urate has only three.

#### *Magnesium ammonium phosphate hexahydrate (Struvite)*

It has been noticed that struvite calculi are fragile, white to dirty white in colour and smooth. Struvite is characterized by a large endotherm at about 160°C and an exotherm at about 690°C. The endotherm seems to be due to the loss of six

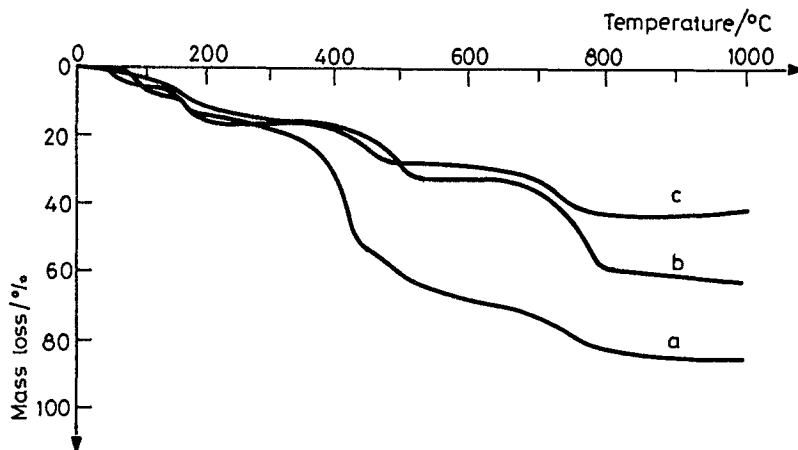
water molecules. The exotherm may be due to the formation of magnesium pyrophosphate from magnesium ammonium phosphate. The above results were confirmed by TG curve given in Fig. 1(c).

IR data reveals that struvite shows a strong broad absorption between  $3300\text{--}3030\text{ cm}^{-1}$  and a shoulder at  $2304\text{ cm}^{-1}$  due to N–H stretching and bending respectively. The very strong and broad absorption between  $1100\text{--}1000\text{ cm}^{-1}$  characterizes phosphate ion. Strong absorption at  $1437\text{ cm}^{-1}$  due to  $\text{NH}_4^+$  deformation vibration and absorptions noted at  $1653$ ,  $1398$ ,  $881$  and  $750\text{ cm}^{-1}$  are characteristic of struvite calculus. This appears to be a composite of the spectra given by  $\text{Mg}(\text{HPO}_4)$  and  $(\text{NH}_4)\text{H}_2\text{PO}_4$  because first exhibits the medium absorption at  $1647\text{--}1645$  and  $881\text{ cm}^{-1}$  whereas second shows a shoulder and medium absorption at  $1408\text{ cm}^{-1}$ .

### Mixtures

DTA results of a stone containing both whewellite and weddellite (Table 1) show that first endothermic peak starts at  $50^\circ\text{C}$  and ends at  $270^\circ\text{C}$  indicating maximum dehydration at  $230^\circ\text{C}$ . This peak shows overlapping of two dehydration process: dehydration of weddellite to whewellite which is converted into anhydrous calcium oxalate. Other peaks are the same as those of whewellite stones.

Figure 3(b) indicates the TG curve for the presence of a mixture of whewellite and weddellite in the same stone. The molecular weights of both hydrates of calcium oxalate have been added and percent weight loss of three molecules of water (17.4%), two molecules of CO (18%) and two molecules of  $\text{CO}_2$  (28.4%) was observed. The values confirmed the presence of both whewellite and weddellite.



**Fig. 3** TG curves of (a) Mixture of Ca-oxalate and Uric acid (b) Mixture of Ca-oxalate (mono and dihydrate) (c) Mixture of Ca-oxalate and Struvite

These results are in good agreement with those presented by Rose [14]. In Table 1 DTA peak positions of a stone containing a mixture of uric acid and whewellite show two endotherms and one exotherm at 200°, 690° and 460°C respectively due to the presence of whewellite and one sharp endotherm at 450°C which corresponds to uric acid. TG curve of a mixture of calcium oxalate and uric acid is shown in Fig. 3(a). The weight loss at 70° to 110°C is due to the loss of water from uric acid. While further weight loss at 140° to 200°C is due to the loss of water of crystallization from calcium oxalate. The weight loss started above 200°C and reached its maximum mode at 450°C which is the characteristics of uric acid. Above 460°C CO escapes and also the loss of uric acid is continued. Carbon dioxide evolves at temperature ranging from 650°–850°C. The DTA and TG results of other mixtures can be interpreted by observing the peak positions and the shape of the curves, respectively.

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**Zusammenfassung** — Mittels TG und DTA wurde die chemische Zusammensetzung von 200 Nierensteinen untersucht, die in den Gebieten Taxila, Rawalpindi und Islamabad in Indien gesammelt wurden. Die Thermogramme zeigen Masseverluste bei verschiedenen Temperaturen, die auf Dehydratation und Zersetzung der Nierensteine hinweisen. Die Ergebnisse wurden mit den qualitativen Resultaten aus der IR-Analyse verglichen, welche die chemische Zusammensetzung der verschiedenen Steine im festen Zustand bekräftigte. Die Thermogramme halfen bei der Unterscheidung von verschieden gebundenem Wasser, ihre chemische Zusammensetzung wurde durch Gewichtsverlust bei der Pyrolyse bestimmt. Man fand, daß 26.5 % der Steine aus reinem Whewellit bestanden, 3 % aus Weddellit, 13 % aus anhydrierter Harnsäure, 7.5 % aus Struvit, 2.5 % aus Ammoniumhydrogenurat, 0.5 % aus Cystin und 47 % der Steine hatten eine gemischte Zusammensetzung. Die häufigsten Kombinationen der Mischzusammensetzungen waren Calciumoxalat mit Harnsäure (14.5 %) und mit Phosphaten (27.5 %).