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TG-FTIR coupled analysis applied to the studies in urolithiasis: characterization of human renal calculi

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Abstract

Many different analytical techniques, as solid state spectroscopy, thermoanalysis, NMR, etc. have been proposed to solve the problems in the field of urolithiasis.

The problems related to these techniques may be overcomed by thermoanalytical methods coupled with the FTIR spectroscopy, that allows to identify and to quantify all the different components of the renal stones. Thermogravimetry coupled with FTIR is a widely technique to characterize renal calculi because of its great sensitivity, reproducibility and accuracy. Moreover, the absence of sample pre-treatments and the short time for the analysis (20–40 min). are very useful for routine purposes. By this method more than 2000 calculi were analyzed.

Keywords: Analysis; Calculi; TG-FTIR; Urolithiasis

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1. Introduction

Urolithiasis is a recurrent disease in many people independently of age or sex. Consequently, many workers have sought informations on human calculi composition to investigate not only the mechanism of calculo-genesis, but also to find preventive measures. For example, if magnesium ammonium phosphate is present, it can be inferred an urinary infection; but if the stratification shows that magnesium ammonium phosphate is only in the outer layers, such infection developed as a complication after the central nucleus aggregation. The alkalinization of the urine is a measure to prevent future recurrence of uric acid calculi, because uric acid and cystine stones are relatively insoluble in acid urine, while quite soluble in alkaline urine; phosphate calculi, in the

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other hand, are relatively soluble in acid urine but insoluble in alkaline urine. Moreover, the introduction of penicillamine for treatment of cystine stones and of allopurinol for uric acid stones (1) has increased the need for the accurate chemical and structural identification of the calculus.

Till from 1949 authors proposed many methods for the analysis of the renal stones, but earlier chemical techniques, then spectroscopic techniques showed their limits. Thermal analysis has been applied to this field (2–10) to solve the problems of spectroscopic techniques when a complex mixture is analyzed, because of the possible interferences among different components contemporary present.

Actually, the possibility to couple Fourier Transform Infrared Spectroscopy (FTIR) with thermal analysis connecting a FTIR instrument to the thermobalance through an hot transfer line allows to solve most of the problems concerning the thermal characterization of the components and their decomposition products (11–13).

Thermogravimetry gives quantitative informations concerning the analyzed calculi and those informations, specially in the case of complex mixtures, are improved by the FTIR spectroscopic analysis of the gases evolved during the thermal decomposition.

The study was carried out characterizing pure substances as: calcium oxalate mono and dyhydrate, calcium phosphate, magnesium and ammonium phosphate, apatites, uric acid and urates, cystine and xantine.

Being extremely rare calculi of indigo, urostealite and sulphonamide, these compounds were not examined.

Synthetic mixtures (as pellets) with simulated composition of the calculi were then analyzed.

Finally, natural human renal calculi derived from surgery, from spontaneous emission and also fragments of calculi coming from lithotrissia treatments were analyzed. The calculi were analyzed after dissection, examination by stereoscopic microscopy and polarized light to identify a possible nucleus which, when present, was isolated and analyzed separately. Fragments were directly analyzed.

To solve the problems deriving from the presence of organic substances, which may be present in the calculi, and whose signals cover those of some thermal resistant compounds, as phosphates or apatites, FTIR was used.

Calculi were submitted to thermal analysis till 1000°C and then the residuals, free of organic substances, were analyzed by FTIR without any interference.

2. Experimental

2.1. Apparatus and reagents

Thermoanalytical curves were obtained using two Perkin Elmer TGA7 thermobalances, for low temperatures (range 20–1000°C) and for high temperatures (range 50–1500°C). The furnace atmosphere was argon, nitrogen or air, with a flow rate of 50–100 ml min^{-1}.

The heating rate was ranged between 5 and 40°C min^{-1}.

The low temperature thermobalance was coupled with a Perkin Elmer FTIR, model 1760X, to obtain the IR spectra of gases evolved during a thermogravimetric analysis. The TGA7 is coupled to the heated gas cell of the FTIR instrument by means of a heated transfer line.

Gaseous or vaporised components are flushed by the suitable gas and the temperatures of the cell and of the transfer line are independently selected.

The only materials in contact with the sample gases are PTFE of the transfer line, KBr of the cell windows and the glass of the TGA/7 furnace.

FTIR spectra of solid systems were carried out using a Perkin Elmer FTIR model 1760X.

The used reagents were supplied by Aldrich Co. and by Merck.

3. Results

Pure substances commonly present in renal calculi were first analyzed by TG-FTIR and the corresponding curves are collected in Figs. 1–7.

TG and DTG curves and FTIR spectra obtained allow the identification of the processes thermally induced and of the corresponding developed gaseous products and are the standard curves against which the examined samples can be compared.

Synthetic mixtures, suitably homogenized, simulating the composition of natural calculi, were prepared by weighing, pelletted and analyzed by TG-FTIR.

Qualitative analysis was carried out by comparing TG, DTG and FTIR curves of the unknown samples with those of the pure substances (14).

Quantitative analysis was carried out by TG curves, which show the weight changes concerning each step of the curve. To each weight change corresponds a decomposition reaction, the stoichiometry of which is known from the TG curves of the pure substances and this allows calculation of the amount of compound in the mixture.

As an example, Figs. 11–12 show the curves corresponding to the synthetic mixtures.

The curves corresponding to some natural calculi are shown in Figs. 13–16.

Sometime, in the temperature range 200–550°C, TG curves show a weight loss which is not present on the standard curves and that can be assigned to the degradation of organic substances present in the calculi.

Increasing the flow rate it was found a sharp improvement of the behaviour of the DTG and FTIR curves with better separation and evidence of the processes while TG curve remain perfectly defined and useful quantitatively.

Figures 8–10 show the FTIR spectra of some phosphates and hydroxoapatite before and after thermal treatment.

4. Discussion

Many analytical techniques have been applied to the analysis of renal calculi because it is very important to know their exact composition either for a correct therapy or to prevent recurrences.

Spectroscopic techniques have been applied to the study of the renal stones: IR spectroscopy, Raman spectroscopy, ESCA, NMR, X Ray diffraction, etc. but the problems related to the interferences of the organic substances were still unresolved.

More recently, new techniques have been proposed, as Small-spot ESCA, proton induced X-ray emission (PIXE), pulsed dye laser (15–18).

These methods are more sensitive than infrared or X-ray spectroscopy, but many problems have been found if organic substances or complex mixtures are present in the stones, as the same authors comment.

Moreover, additional techniques are recommended to support the results.

Methods based on thermoanalytical techniques (also using IR spectroscopy as ancillary technique) have been proposed to obtain reliable results (19). Thousands of analysis carried out in more than ten years have shown that sometimes doubtful data are obtained specially concerning:

- very complex mixtures so that some decomposition processes tend to be superimposed
- calculi containing organic substances (specially blood) and phosphates or hydroxoapatite, because TG signals are weak, or absent as in the case of apatite and the IR signals are covered by those concerning the organic substances.

Qualitative data obtained by thermoanalytical techniques are remarkable improved by the informations obtained from the FTIR spectra concerning the gases deriving from each thermal decomposition.

As shown by the FTIR spectra of the thermodecomposition of the pure substances only few sharp and specific bands appear for each process.

For some substances thermal decomposition gives rise to only one chemical product for each step as in the case of calcium oxalate mono and dyhydrate, whose thermoanalytical curves show the three classical demolition steps corresponding, in non oxidant atmosphere, to the loss of H_2O, CO and CO_2 respectively (Fig. 1).

Beside to the water signals, the CO_2 bands are localized at 2350 cm^{-1} (stretching) and 670 cm^{-1} (bending).

Concerning the uric acid and urates, whose thermal decomposition occur in only one step (Figs. 2, 3), FTIR spectra show bands at 966 and 931 cm^{-1} ascribing to the NH_3 and one band with two peaks at 3529 and 3515 cm^{-1} ascribing to the R-NH_2, besides the signals concerning CO_2.

Decomposition of standard cystine is referred in Fig. 4 and the IR spectrum shows the characteristic bands of CO_2 and NH_3. Moreover, the band at 3335 cm^{-1} is related to the NH_3 as a A_1 total symmetric band. The band at 2973/2929 cm^{-1} is ascribed to the ν_{as} of a CH_2=CH_2 fragment, the band at 1625 cm^{-1} is due to a R-NH_2 in plane bending while the bands in the broad region 1560/700 cm^{-1} can be attributed to vibrations involving interactions between C=S stretching and C–N stretching.

The magnesium ammonium phosphate hydrate shows the characteristic bands of water and ammonium (Fig. 5).

Standard xantine (Fig. 6) decompose in only one step and shows the bands at 2350 and 670 cm^{-1}, 966–931 and 3334 cm^{-1}, 3530–3510 cm^{-1}.

The analysis of standard phosphate, $Mg_3(PO_4)_2$, $Ca(HPO_4)$, $Ca(H_2PO_4)_2$ was carried out heating phosphates till 1000°C by TG (Fig. 7) and recording IR spectra of

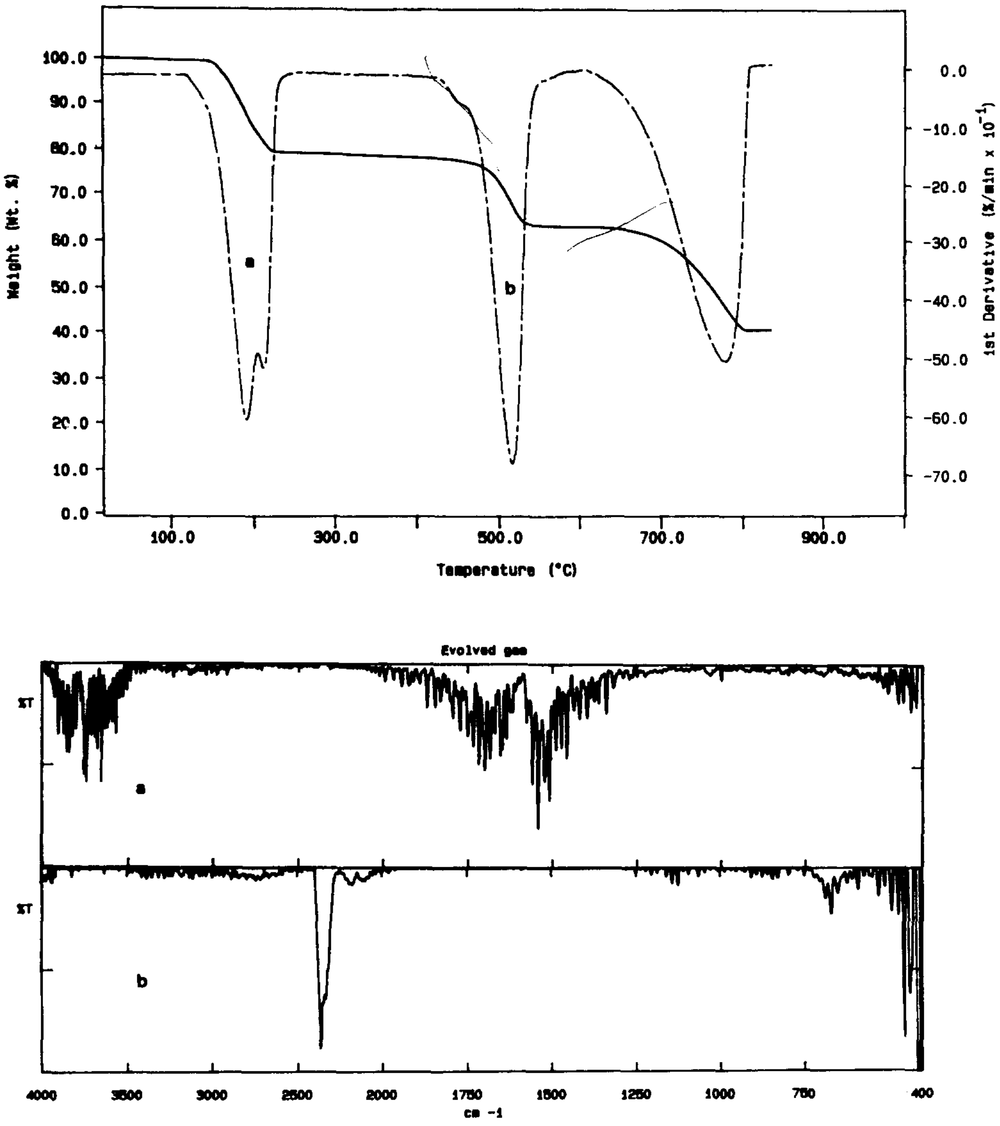

Fig. 1. TG and DTG curves of Calcium oxalate dihydrate. Heating rate 20°C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

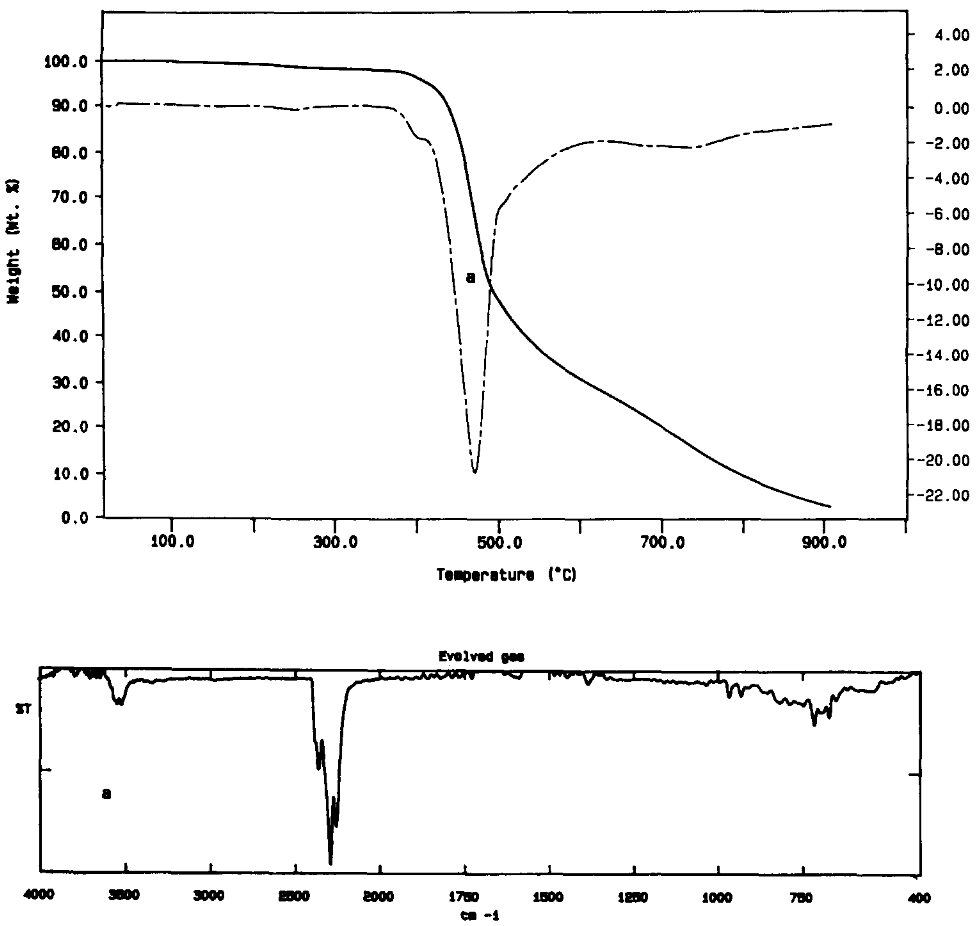

Fig. 2. TG and DTG curve of Uric acid. Heating rate 20°C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

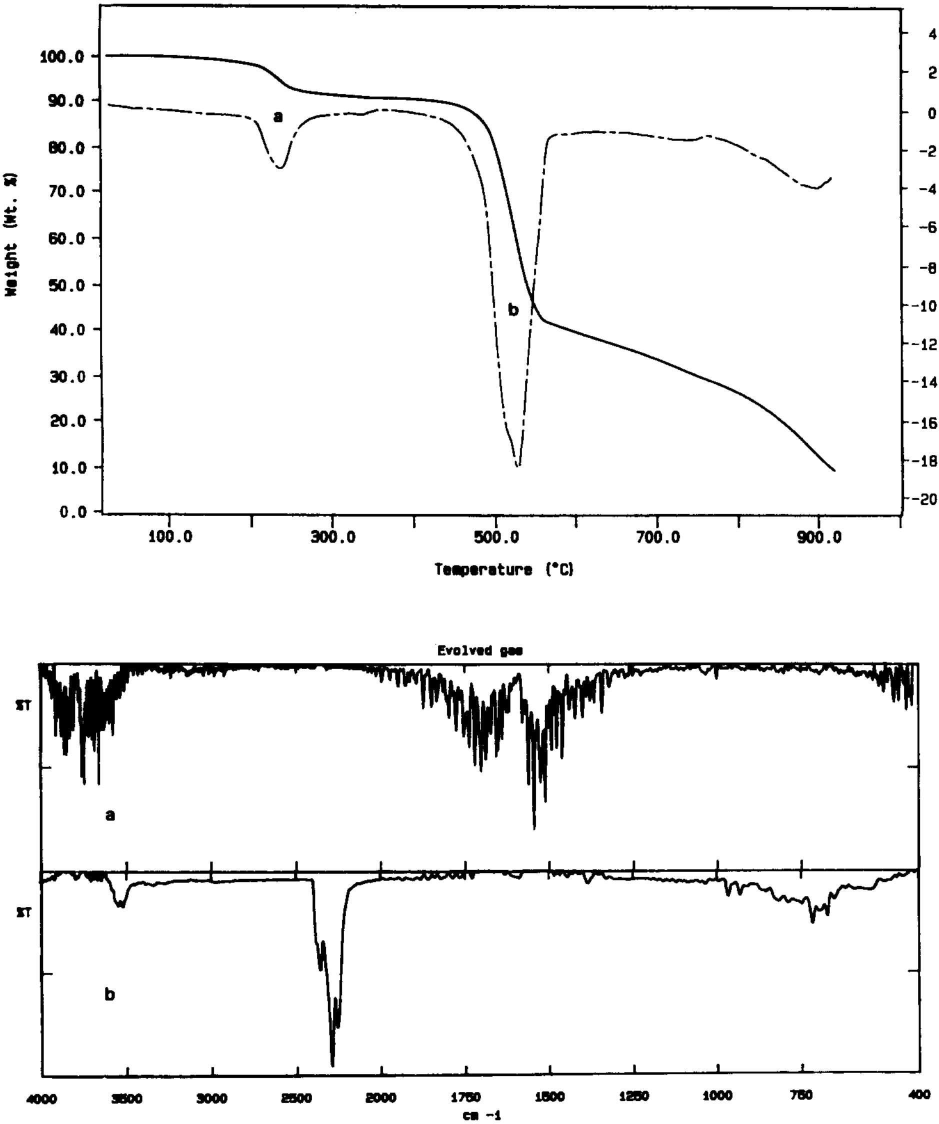

Fig. 3. TG and DTG curve of monosodic salt of uric acid (monohydrate). Heating rate 20°C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

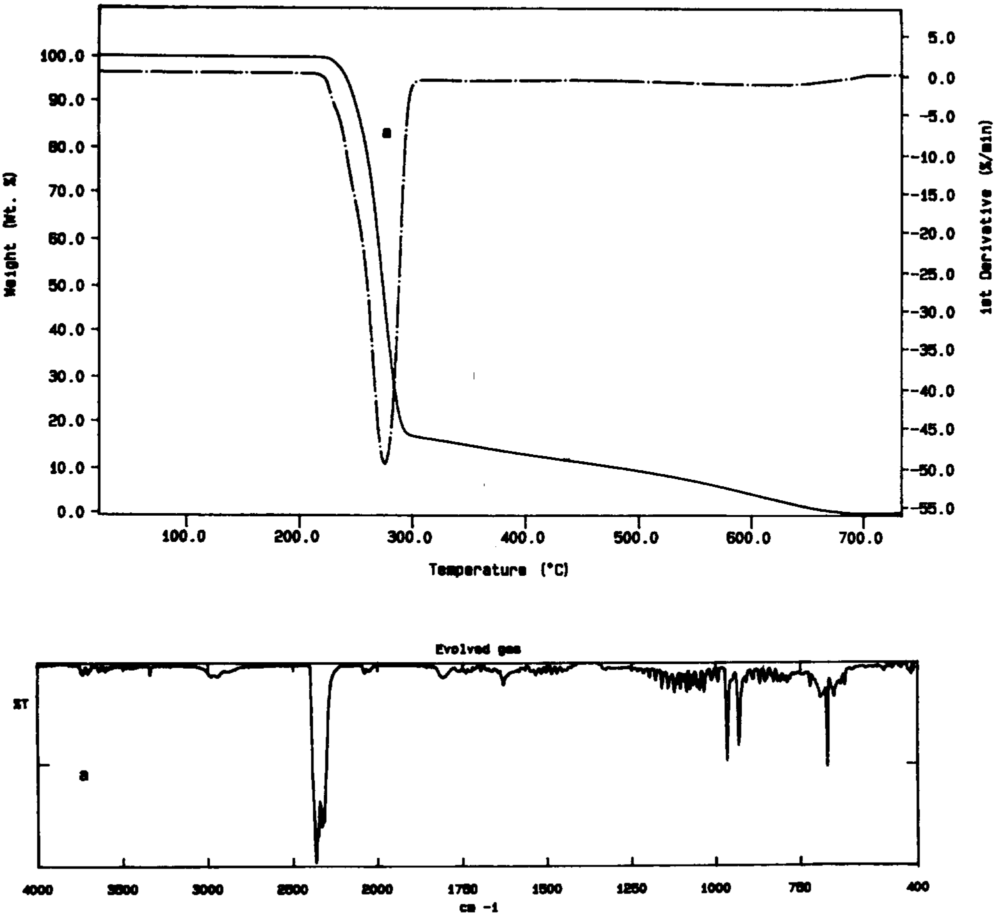

Fig. 4. TG and DTG curve of Cystin. Heating rate 20°C $\min^{-1}$. Atmosphere: air at a flow rate of 100 ml $\min^{-1}$.

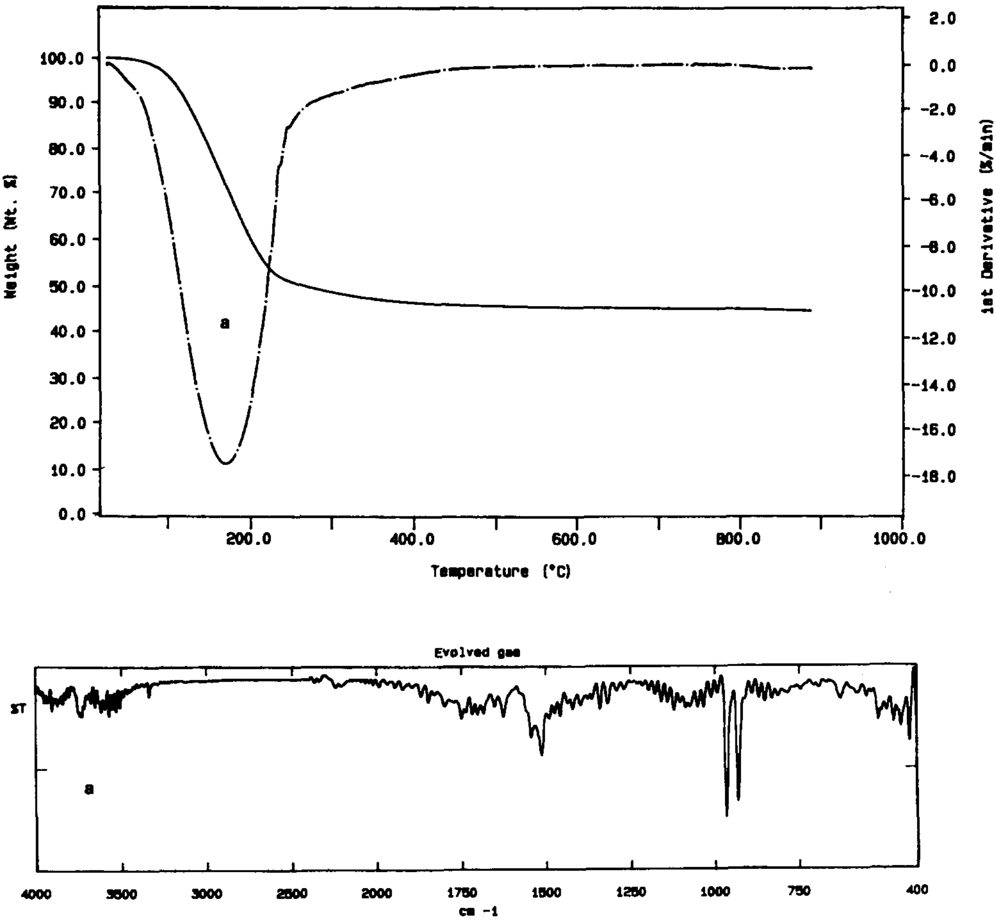

Fig. 5. TG and DTG curve of Ammonium magnesium phosphate hexahdrate. Heating rate 20°C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

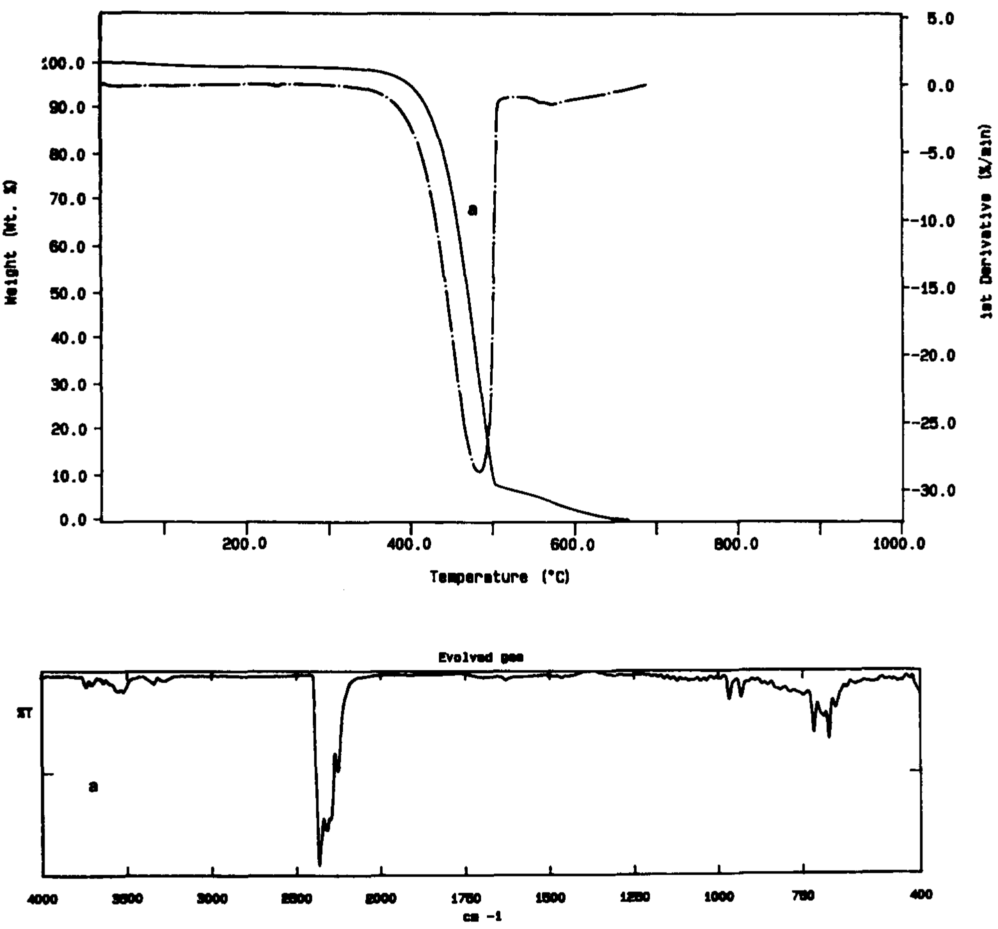

Fig. 6. TG and DTG curve of Xantyn. Heating rate 20°C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

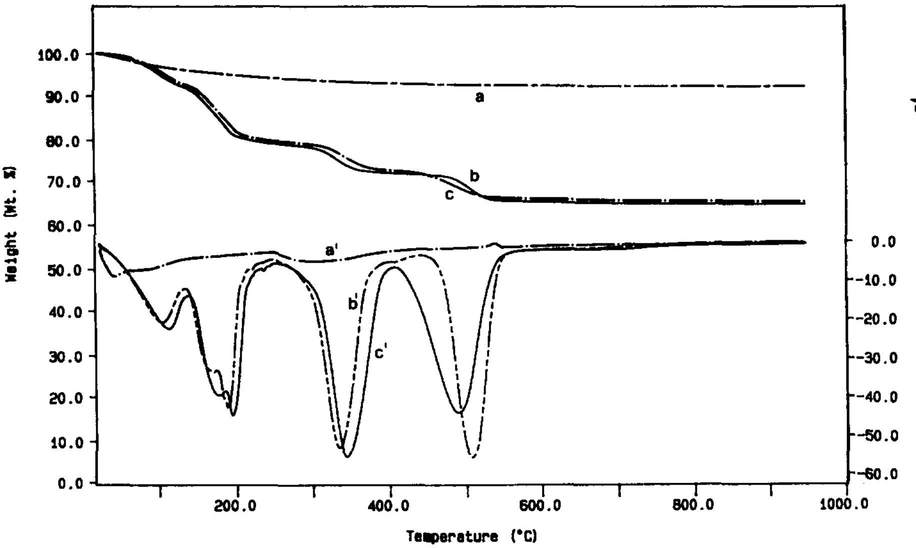

Fig. 7. TG and DTG curves of: (a/a') $Mg_3(PO_4)_2$; (b/b') $CaHPO_4$; (c/c') $Ca(H_2PO_4)_2$. Heating rate 20°C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

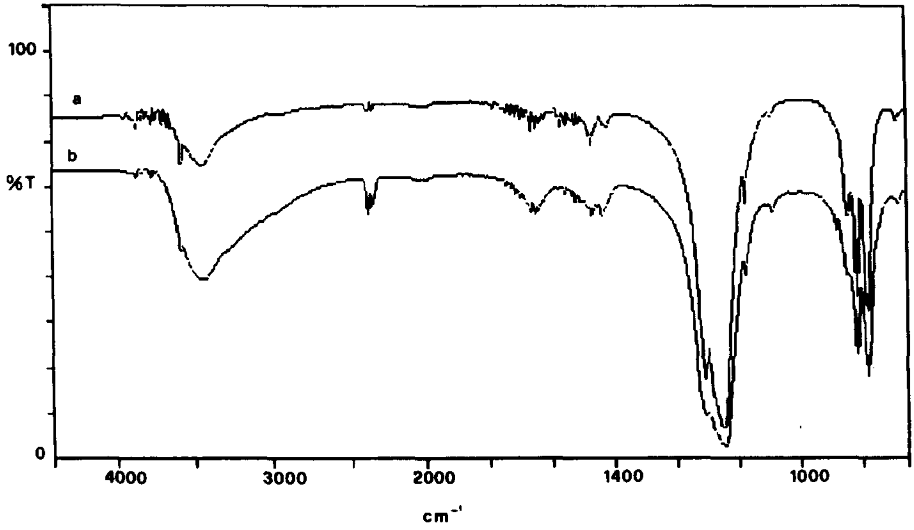

Fig. 8. FTIR spectra of phosphate (PO_4): (a) before and (b) after heating. Resolution 4 cm^{-1}, 20 scans.

the samples, in KBr, before and after heating, to compare the IR bands also after thermal treatment.

TG analysis of phosphates is difficult because the thermochemical processes are of low intensity or completely absent. Better results can be obtained from IR spectra, but organic substances are commonly present and mask the characteristic bands.

The spectra of the standard phosphates before and after heating at 1000°C (therefore after the loss of organic substances) show that the bands at 3490 and 1050 cm^{-1} are present and reproducible before and also after heating of phosphate, so we can take them as diagnostic (Figs. 8–10).

Figs. 11 and 12 show the curves of two mixtures choosen as example.

From the characteristic decomposition temperatures and the FTIR spectra of the evolved gas, it is confirmed the composition:

mixture A = magnesium ammonium phosphate 50% – xanthine 50%

mixture B = oxalate monohydrate 19% - uric acid 70% – apatite 11%.

The usefulness of this coupled technique is shown by the Figs. 13–16, where are reported the curves and the spectra of a little number of natural calculi, being the more representative of the pathologic situations among the samples analyzed.

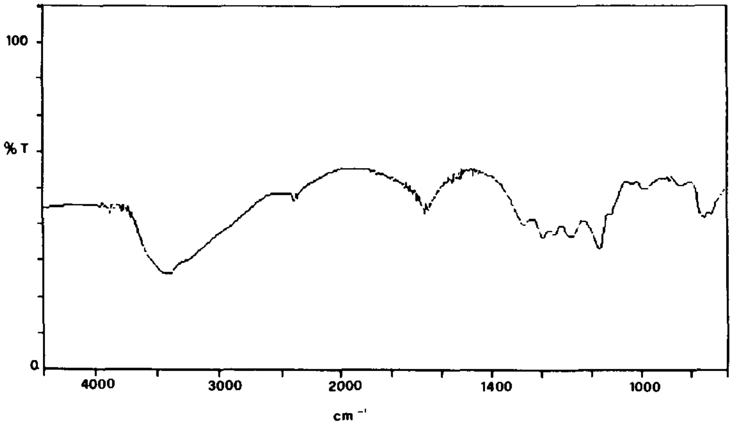

Fig. 9. FTIR spectrum of phosphate (HPO_4) after heating. Resolution 4 cm^{-1}, 20 scans.

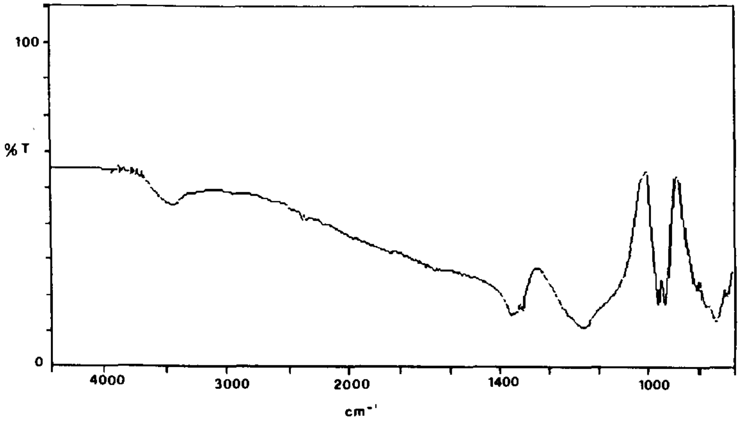

Fig. 10. FTIR spectrum of phosphate (H_2PO_4) after heating. Resolution 4 cm^{-1}, 20 scans.

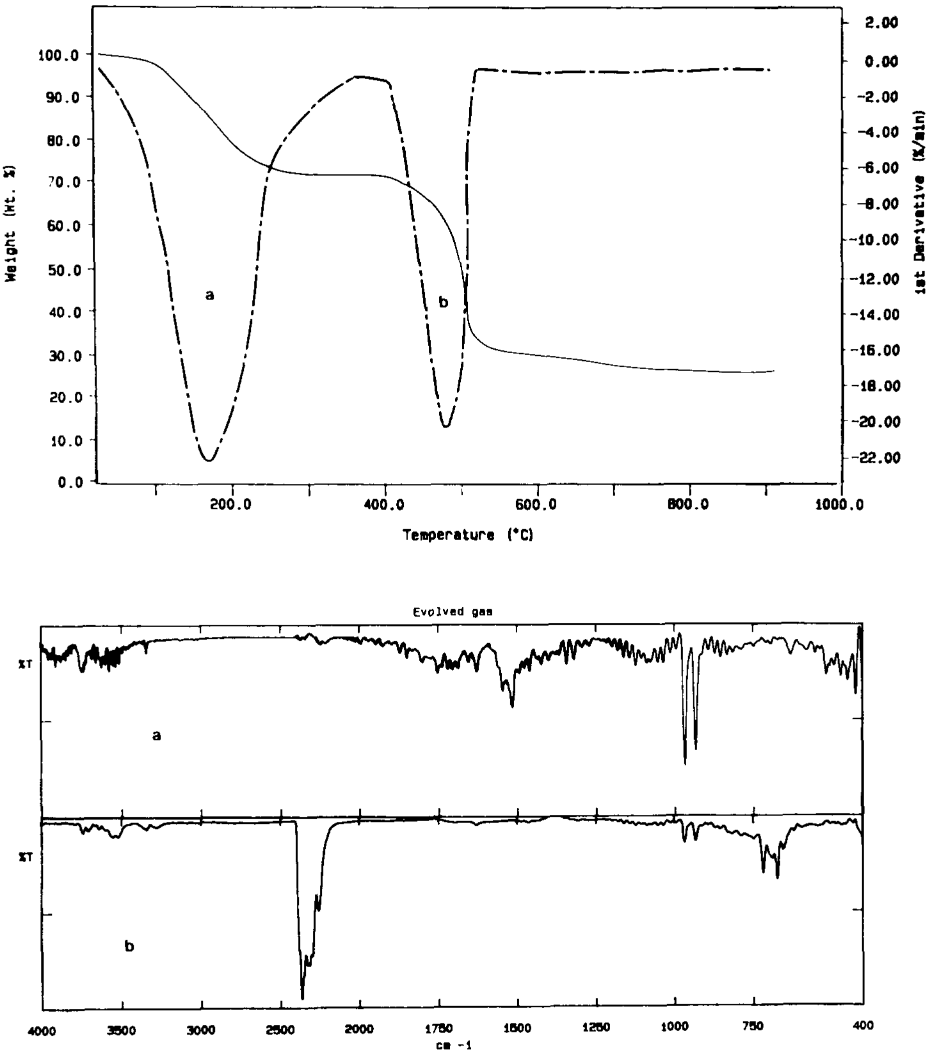

Fig. 11. TG and DTG curve of the synthetic mixture A. Heating rate 20°C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

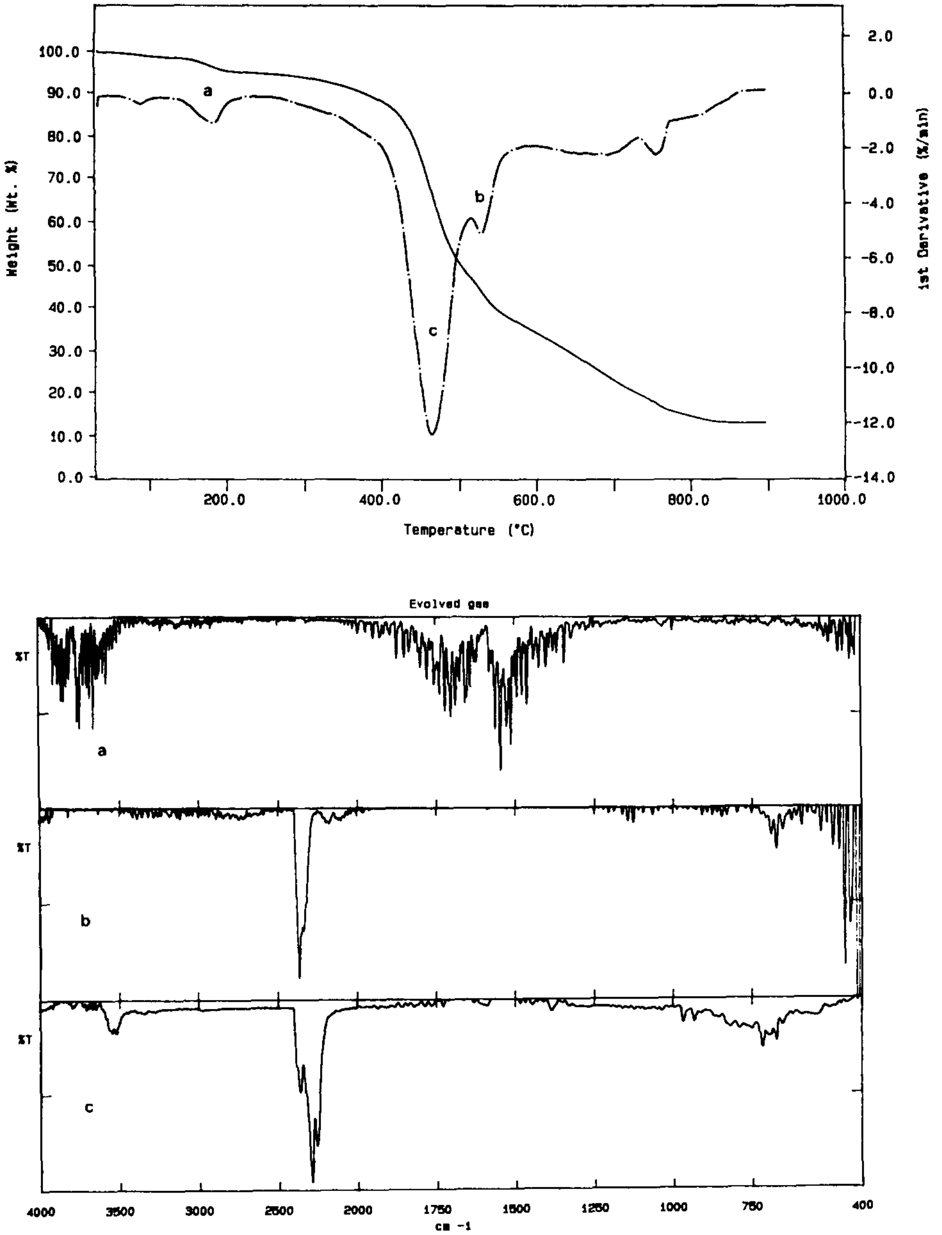

Fig. 12. TG and DTG curve of the synthetic mixture B. Heating rate 20°C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

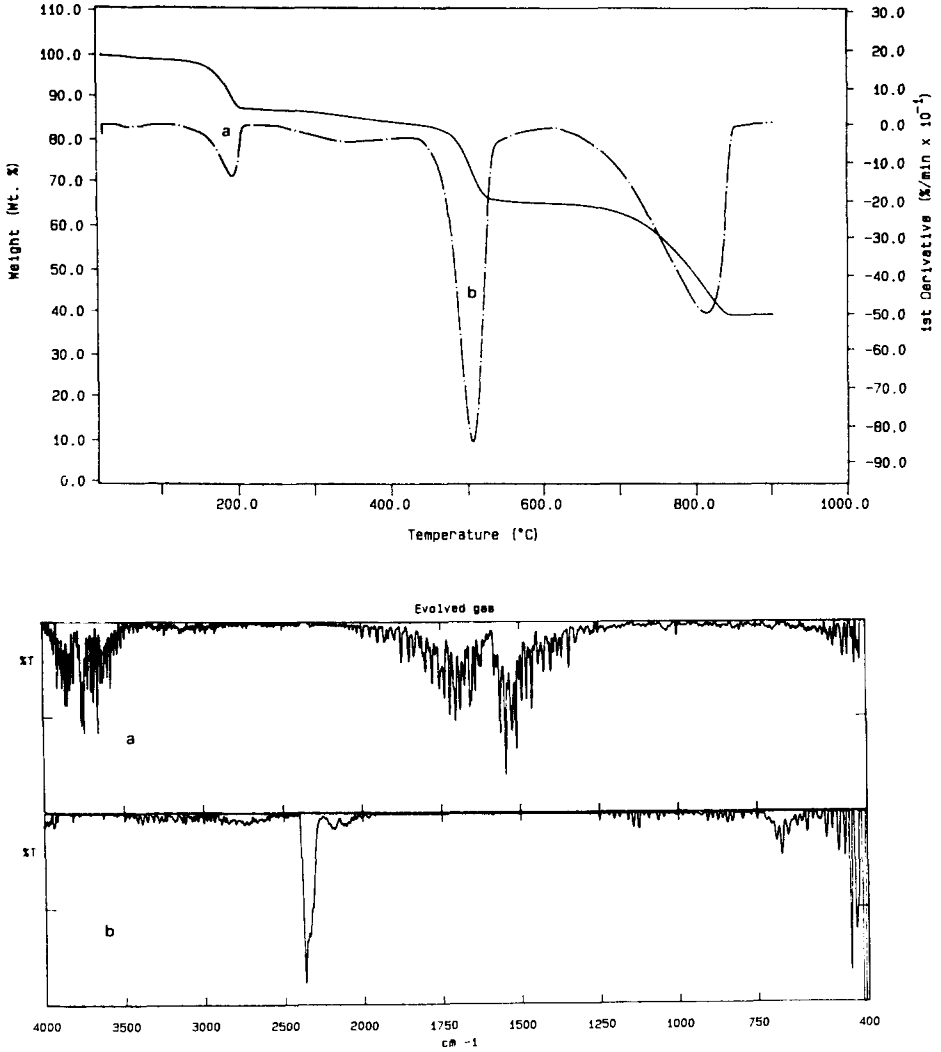

Fig. 13. TG and DTG curves of human renal calculus. Heating rate 20°C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

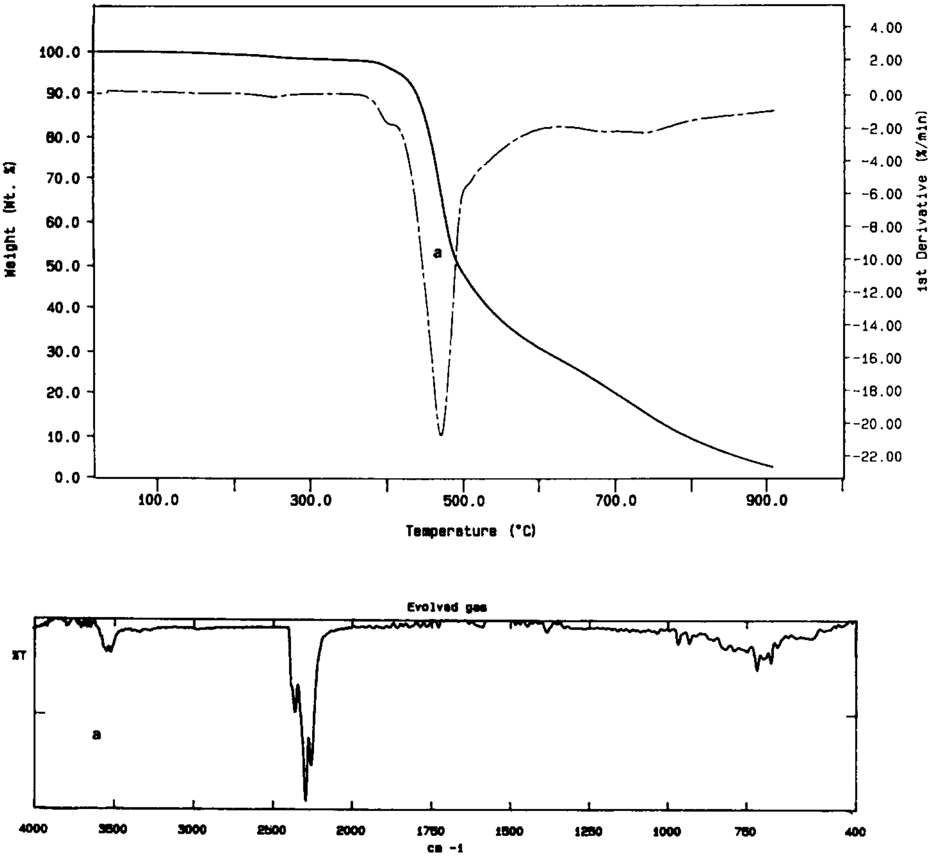

Fig. 14. TG and DTG curves of human renal calculus. Heating rate 20°C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

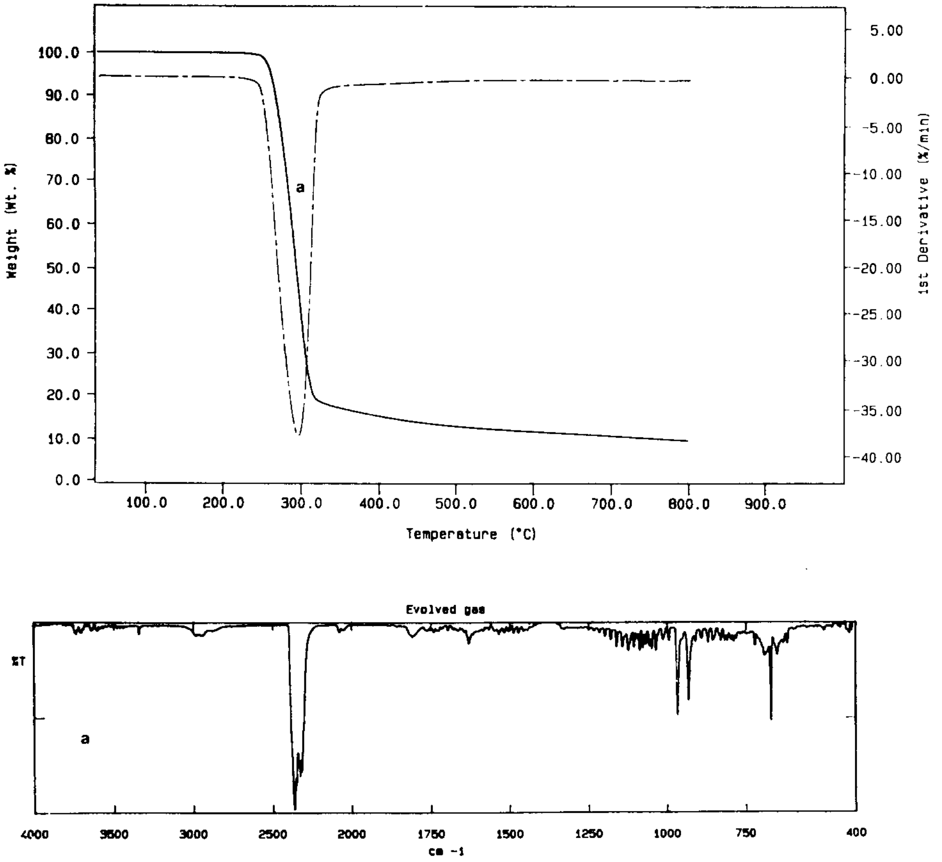

Fig. 15. TG and DTG curves of human renal calculus. Heating rate 20°C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

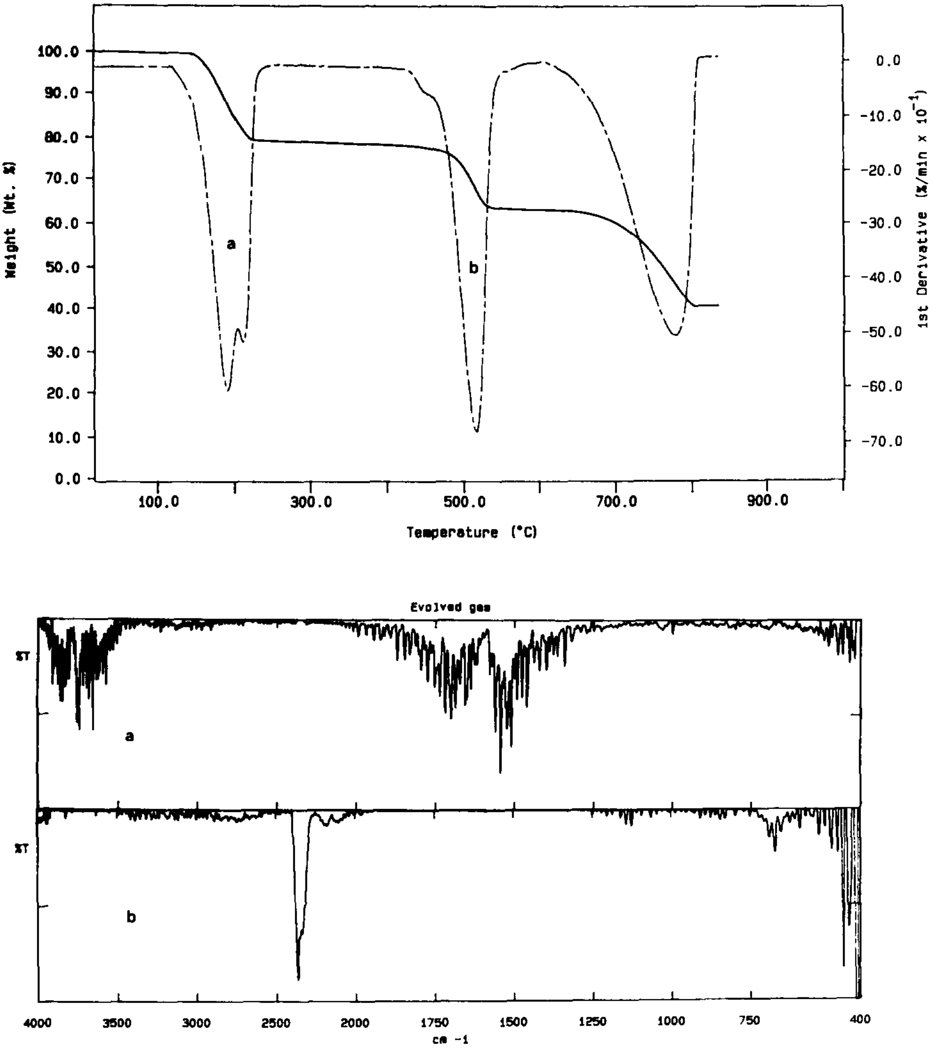

Fig. 16. TG and DTG curves of human renal calculus. Heating rate 20°C min^{-1}. Atmosphere: air at a flow rate of 100 ml min^{-1}.

By comparing TG characteristic temperatures and FTIR spectra of the evolved gas obtained for the standard substances, we can assign:

Fig. 13 renal calculus of composition oxalate dyhydrate 92% – apatite 8%
Fig. 14 100% uric acid calculus
Fig. 15 calculus of composition cystine 90% – apatite 10%
Fig. 16 calculus of composition oxalate monohydrate 83% – apatite 17%

Thermoanalytical techniques are a tool of high potential in the field of nephrolithiasis, allowing the realisation of an analytical method useful not only in obtaining the quantitative analysis of the chemical species present in the calculus, but also in determining the crystalline structure of the chemical species present in the calculi, knowledge which is fundamental for a correct therapeutical treatment, or the hydration state of the salts that constitute the nephrolites.

The proposed analytical method allows the realisation of the analysis of a calculus, especially using high speeds of temperature increase, in very short times, also useful for routine purposes. The method does not require sample pretreatment, is simple and has an accuracy of about 1.5%.

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