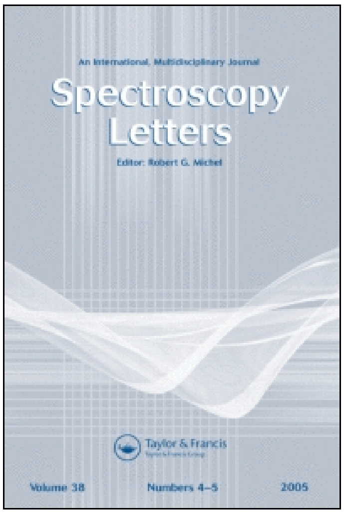

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Infrared Analysis of Urinary Stones, Using a Single Reflection Accessory and a KBr Pellet Transmission*

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ABSTRACT This work is a comparative study of two FTIR techniques applied to urinary stones analysis: single reflection diamond attenuated total reflection (ATR) and KBr pellet transmission (KPT). We show that the two methods allow the identification of all stone components. The ATR technique is more useful and rapid to identify the species without sample pretreatment. Nevertheless, KPT is more appropriate for components determination in urinary stones.

These techniques were applied to the study of a series of 313 calculi. The stone constituents were first identified by ATR, and in a second step, the proportion of each species present in the stone was determined by KPT in whole-stone mixture.

The results obtained showed the presence of 11 different components classified as follows with the frequency of detection in the stones studied: calcium oxalate monohydrate (whewellite), 78.9%; carbapatite, 33.9%; calcium oxalate dihydrate (weddellite), 24%; uric acid anhydrous, 19.2%; ammonium hydrogen urate, 7%; struvite, 4.8%; cystine, 1%; ammonium sodium urate and other phosphates (amorphous carbonated calcium phosphate, brushite, whitlockite), each in less than 1%.

KEYWORDS ATR, infrared spectroscopy, transmission, urinary stones, urinary stones constituents

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1. INTRODUCTION

Urolithiasis is a frequent disease that affects between 3% and 20% of the population in industrialized countries with a recurrence after treatment in 50%.[1–3] This disorder is also in progression worldwide touching the two sexes at any age. This afflication can lead to end-stage renal failure leading to chronic hemodialysis. These data show the importance of stones analysis to improve therapy and to avoid multirecurrent nephrolithiasis. The determination of crystalline and molecular composition and the quantification of all stone components are necessary to establish the etiology of stones disease.

The identification of components in the stones can be performed by wet chemical methods or by physical techniques such as crystallographic methods,[4,5] thermal analysis,[6] and Fourier transform IR (FTIR) or Raman spectroscopy.[7,8] Chemical analysis is relatively inaccurate because of false-positive and false-negative results and does not allow distinguishing between the crystalline phases.[9] Among physical methods, X-ray diffraction is appropriate for quantification of mineral samples, but it cannot adequately detect amorphous species such carbapatite or struvite.[7,9]

FTIR spectroscopy is the most appropriate technique for stones analysis. Moreover, the combination of this technique with optical microscopy allows a morpho-constitutional characterization that leads to pathogenic origin and identification and quantification of the different components in different parts of the stones.[7,8,10]

The aim of this study was to propose a complete procedure for stones analysis including a rapid qualitative study by single reflection diamond attenuated total reflection (ATR) and an accuracy quantitative analysis of the different components of stones by KBr pellet transmission (KPT). These two techniques were then applied to study a series of 313 stones in order to identify and quantify the components detected.

2. MATERIALS AND METHODS

2.1. Materials

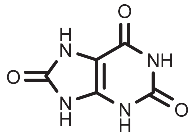
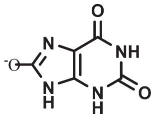
2.1.1. Instrumentation

The FTIR spectrometer used was a JASCO FTIR 460 PLUS (Pike Technologies, Madison, USA), and the ATR accessory was a Diamond Miracle TM single reflection Horizontal Attenuated Total Reflectance (HATR).

2.1.2. Samples

- Powders of calcium oxalate monohydrate (whewellite; WHE), calcium oxalate dihydrate (wedellite; WED), uric acid anhydrous (UAA), ammonium hydrogen urate (AHU), ammonium sodium urate (ASU), cystine (CYS), carbapatite (CARB), struvite (STR), amorphous carbonated calcium phosphate (ACCP), brushite (BRU), whitlockite (WHIT) sampled from patients stones of known composition. Table 1 shows mineralogical and chemical characteristics of these components.
- A series of 313 stones collected from patients in the Rabat Salé region of Morocco.

TABLE 1 **Chemical and Mineralogical Features of the Main Stone Components**

Stone component	Mineralogical title	Chemical formula
Calcium oxalate monohydrate	Whewellite	$CaC_2O_4 \cdot H_2O$
Calcium oxalate dihydrate	Weddellite	$CaC_2O_4 \cdot 2H_2O$
Carbonate apatite	Carbapatite, dahllite	$Ca_{10}(PO_4)_6CO_3$
Magnesium ammonium phosphate hexahydrate	Struvite	$NH_4MgPO_4 \cdot 6H_2O$
Uric acid anhydrous	Uricite	
Urates	—	
Cystine	—	$(-S-CH-CHNH_2-COOH)_2$
Calcium acid phosphate dihydrate	Brushite	$CaHPO_4 \cdot 2H_2O$
Calcium magnesium phosphate	Whitlockite	$(CaMg)_2(PO_4)_2$

2.2. Methods

As known, IR spectroscopy is managed following several sampling techniques depending on the nature of the sample. Two sampling techniques were used in our study:

- Single reflection diamond ATR: The spectra of the components were obtained without any sample preparation; the stone sample was placed directly on the ATR cell. The sample was pressed onto the diamond surface, and the spectrum was recorded from 4000 to 600 cm^{-1}.
- KBr pellet transmission: The sample was pulverized then diluted in dried KBr to obtain a homogeneous mixture using a mortar and a pestle made of agate. The mixture was placed in an appropriate mold and pressed at 10 tons/cm^2 to form a semitransparent pellet 13 mm in diameter. The pellet was placed in the IR beam using the sample transmission holder. The spectra were performed in middle IR from 4000 to 400 cm^{-1}.

The first part of this work was reserved to record the spectra of the most commonly occurring components of urinary stones by ATR and KPT and to compare each pair of spectra obtained by these techniques.

Spectra of the constituents were obtained by single reflection diamond ATR and KPT using pure compounds isolated from stone samples of a known composition.

With ATR, samples were analyzed without pretreatment; in KPT, the samples were diluted to 1% in dried KBr and made into pellets.

The second part of this work consisted of a study of a series of 313 stones in order to determine morphologic features by optical microscopy, then the identification and the quantification of crystalline and noncrystalline stone species respectively by ATR and KPT.

2.3. Morphologic Study of Stones

The first operation was the examination of each stone under a microscope to distinguish different layers and to pick up some amount for analysis by IR spectroscopy. The characteristics of the stone structure (layers, nucleus, crystallization phases, color, form, etc.) offer some indications on the nature of components and make easy the identification of the components with the IR spectra.

2.4. Sampling and IR Spectroscopy Analysis of Stones

2.4.1. Identification of Components in Stones

The identification was easily performed by ATR. The sampled parts of different zones were picked up in approximately the same amount. For each zone was recorded a spectrum by ATR to determine the component present without any preparation or transformation of sample as previously described.

2.4.2. Quantification of Components

The proportion of each component in a particular stone was obtained by analysis of the whole-stone powder.

Usually no more than three components can be detected in one stone. The majority of all urinary stones contain one, two, or maximum three components.[7,10] The Beer–Lambert law was applied for the semiquantitative determination of each component when the stones were of mixed composition.[7,8] The

TABLE 2 Characteristic Wave Numbers of Main Stones Species

Stones constituent	Characteristic bands (cm^{-1}) of quantitative interest
Whewellite	948; 1620; 3490
Weddellite	912; 1640
Carbapatite	601; 1035
Uric acid anhydrous	745; 3010
Ammonium hydrogen urate	770
Struvite	570; 1000

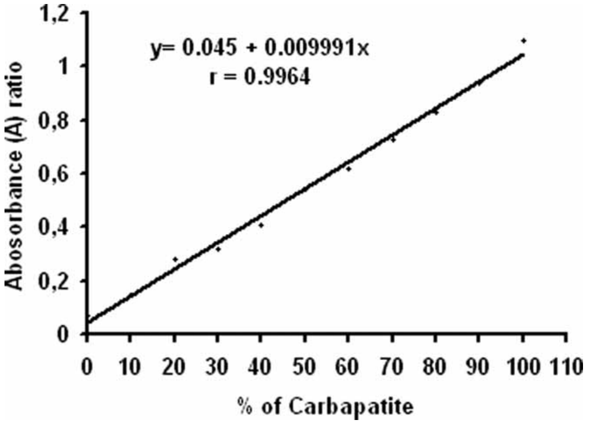


FIGURE 1 Standard Curve of Carabapatite-Struvite Mixture; Absorbencies Ratio Measured at 600/570 cm.$^{-}$

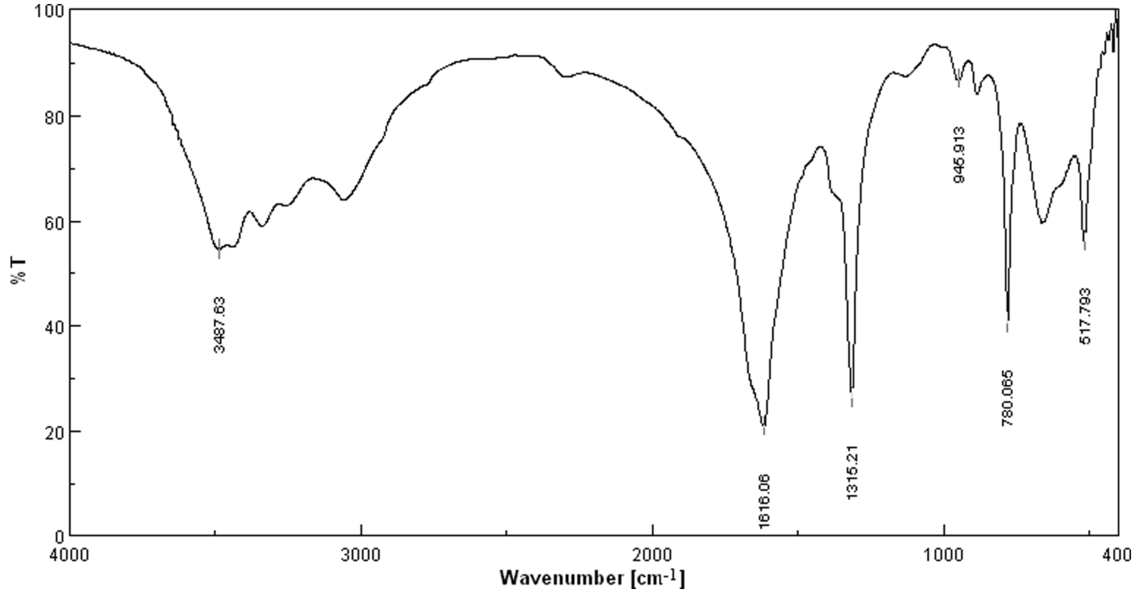


FIGURE 2 IR Spectrum of Pure Whewellite.

quantitative determination is carried out by measuring absorbance at the characteristic band of each stone component in the corresponding IR spectrum. Table 2 shows the main bands of the most common stones constituents.

The band was selected depending on the other specie(s) present in the stone: when WHE and WED are associated, the absorbance is measured respectively at 946 cm^{-1} and 915 cm^{-1}. In a mixture with the previous species and UAA, the detection is managed respectively at 3490 cm^{-1}, 915 cm^{-1}, and 3010 cm^{-1}. The ratio of the absorbencies of the characteristic absorption peaks corresponding with each binary mixture was calculated using the IR spectrum of the mixture, and the percentage was inferred from the calibration curve. Calibration curves used were realized using OPUS, a computer program provided by Bruker Spectrospin. More than 5000 spectra of

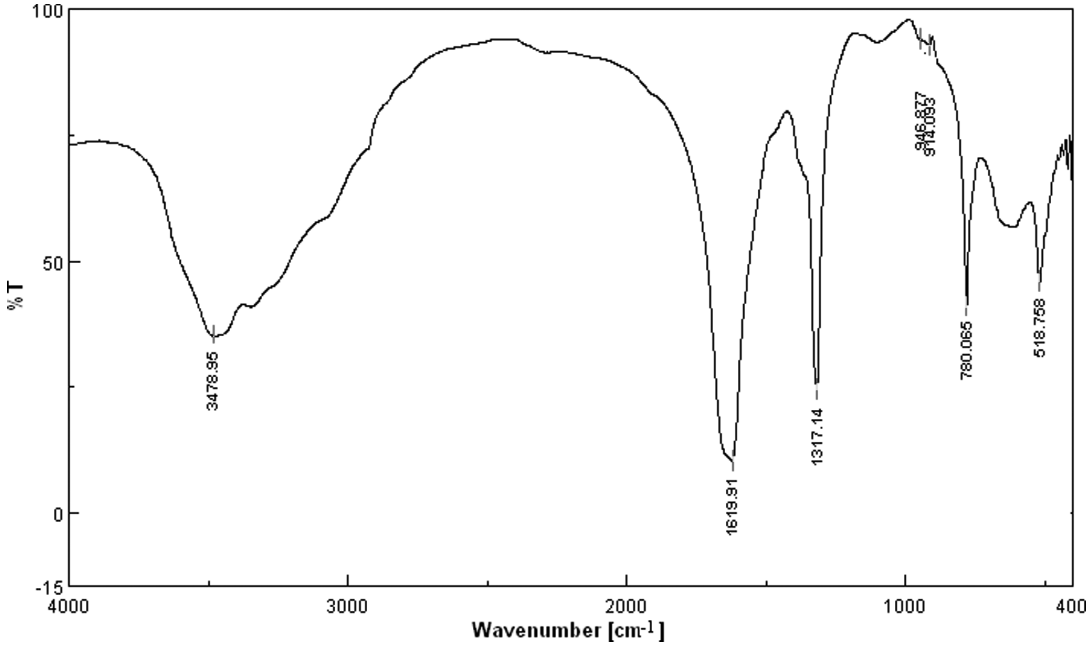


FIGURE 3 IR Spectrum of Whewellite and Weddellite Mixture.

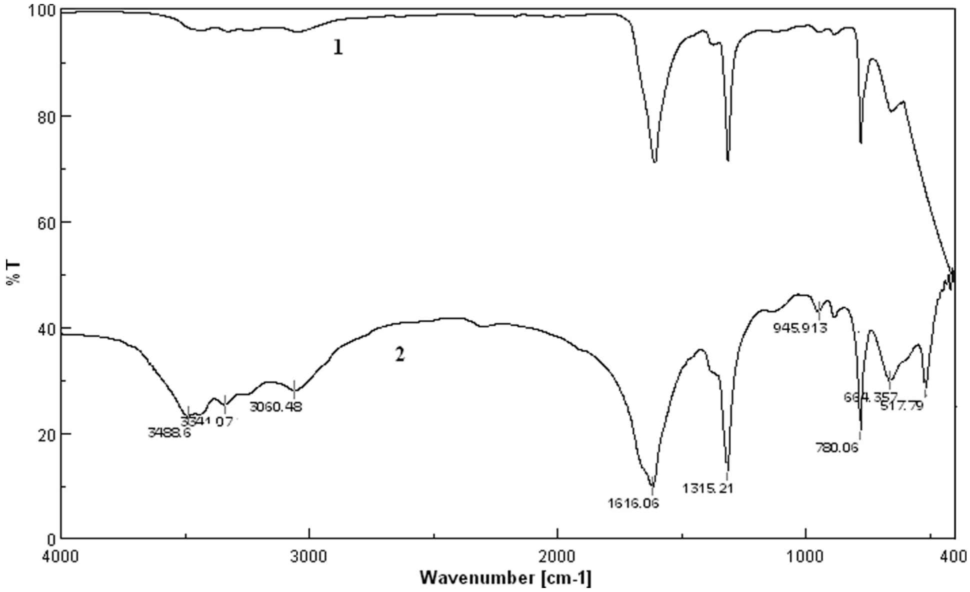


FIGURE 4 Spectra ATR (1) and KPT (2) of Whewellite.

binary and ternary mixtures were mathematically recorded from spectra of pure stones using the additivity of absorbance. Each mixture was prepared in linear ranges of 10–90% with step sizes of 10%. Curves were expressed as

$$A = A_1\nu_1/A_2\nu_2 = \mathrm{f}(X\%)$$

where ν_1 and ν_2 are the two wave numbers selected, A_1 and A_2 are the respective absorbancies, and X is the proportion of one of the two components. Figure 1 shows the standard curve of a CARB/STR performed using a series of mixtures respectively at proportions of 0–100% with a step of 10%.

When stones contain more than three components, the respective proportion of constituents was extrapolated from those found using calibration curves for binary or ternary mixtures.

The FTIR spectrometer was validated by measurement of polystyrene transmission standard to check wave number positions and absorbencies of known IR bands. The suitability system was also checked by measuring transmittance and absorbance repeatability,

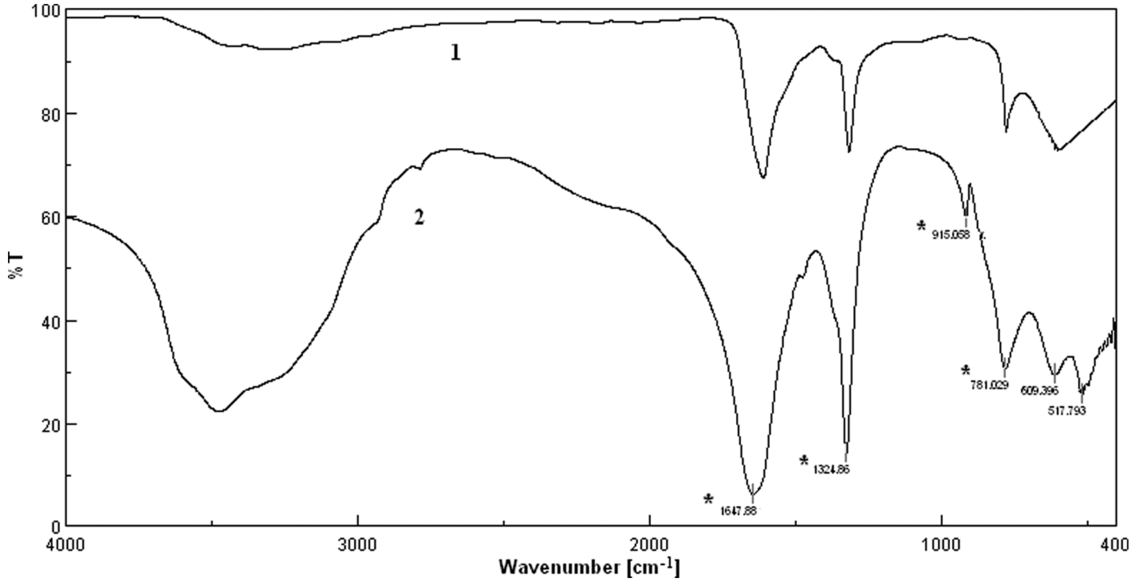


FIGURE 5 Spectra ATR (1) and KPT (2) of Weddellite.

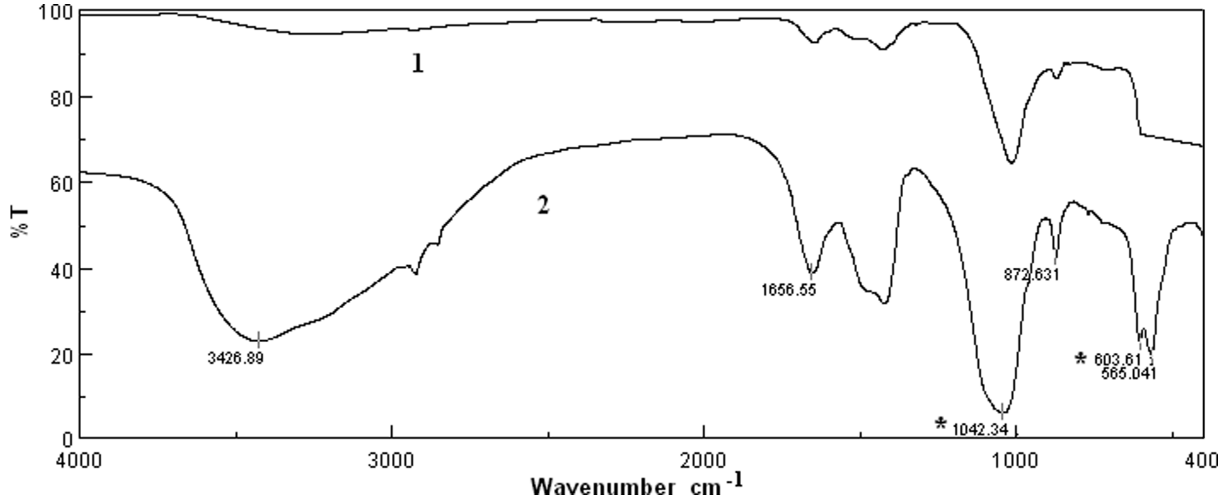


FIGURE 6 Spectra ATR (1) and KPT (2) of Carbapatite.

100% transmittance line, the noise intensity, and the resolution.

3. RESULTS AND DISCUSSION

3.1. IR Spectra of Some Components

Each stone component is characterized by its proper spectrum with absorption bands (stretching or bending) representing specific molecular composition and crystal structure. All spectra were identified by comparison with reference IR spectra of pure compounds using the characteristic peaks and the key bands when species were in mixture. The WHE is characterized by a strong band C=O at 1620 cm^{-1} asymmetric to the left, a moderate band C-O near 1315 cm^{-1}, and a valence vibration of H_2O in staircase form from 3500 to 2700 cm^{-1} with two maxima at 3498 and 3440 cm^{-1} (Fig. 2). In the fingerprint region, the characteristic bands are an out-of-the-plane deformation band at 781 cm^{-1} and three secondary vibrations at 515, 885, and 946 cm^{-1}. The key bands are 780; 1315; 1620; 3498 and 3440 cm^{-1}. In a WHE and WED stone mixture, the characteristic bands are respectively 915 and 946 cm^{-1}, in addition, the C-O and C=O

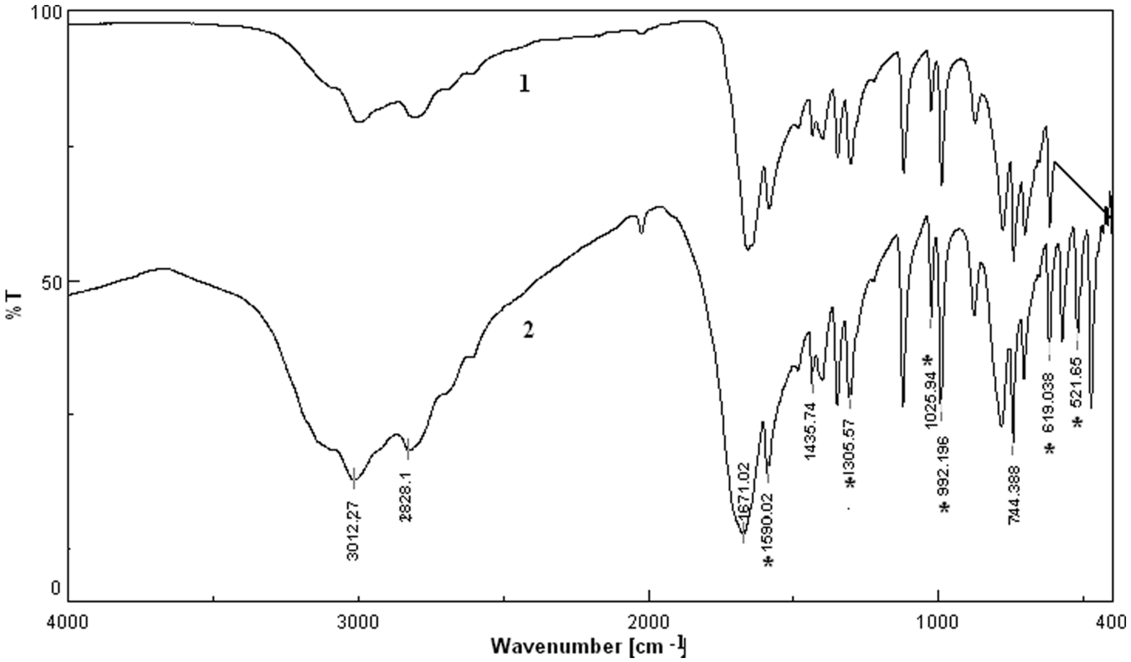


FIGURE 7 Spectra ATR (1) and KPT (2) of Uric Acid Anhydrous.

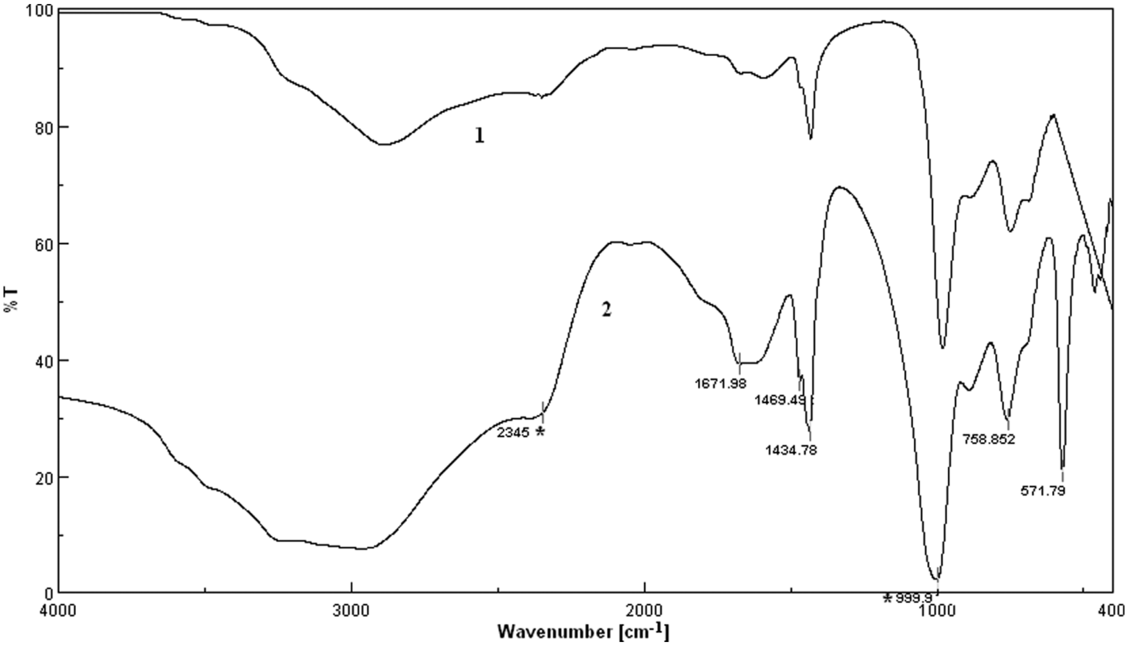


FIGURE 8 Spectra ATR (1) and KPT (2) of Struvite.

bands are shifted in the left and the band at 781 cm^{-1} is relatively less intense and very broad (Fig. 3).

The examination of the spectra in Figures 4 to 11, shows that in general, the ATR spectra are relatively of low intensity, and no information is given on the side of 650 cm^{-1}. Therefore, the characteristic bands in this region are not available, and no quantification is possible: For example, the determination of CAR and STR in mixture is managed respectively at 570 cm^{-1} and 600 cm^{-1}; in the same case when STR is in mixture with UHA, the two components were determined respectively at 770 cm^{-1} and 570 cm^{-1} (the last peak is not available on ATR spectrum).

3.2. Patients' Stones Study

As described previously, the identification was performed by ATR and the content of constituents in

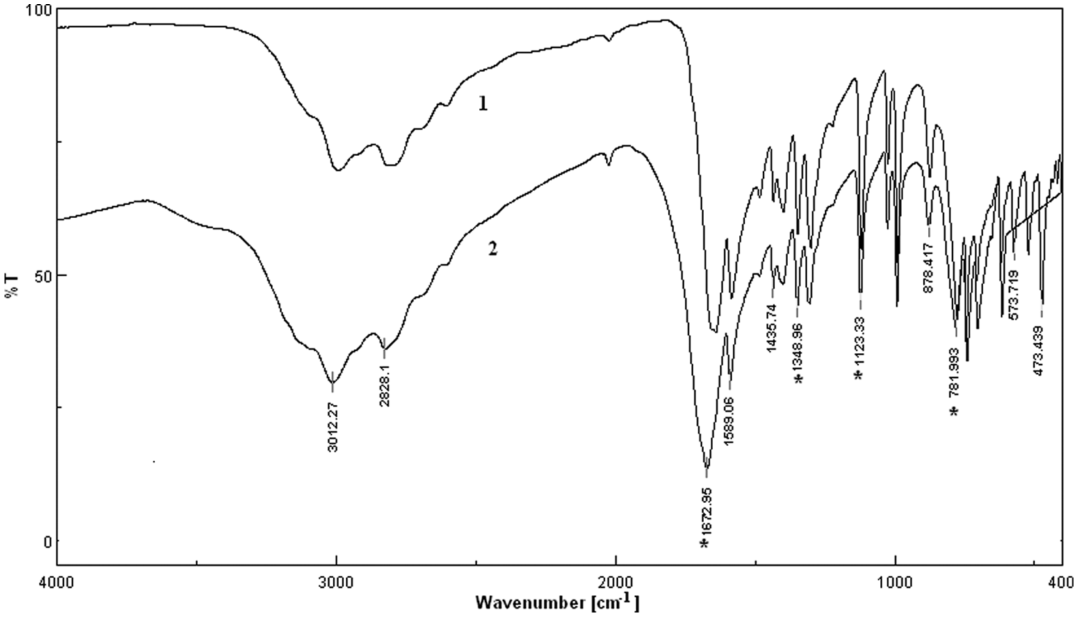


FIGURE 9 Spectra ATR (1) and KPT (2) of Urate Hydrogen Ammonium.

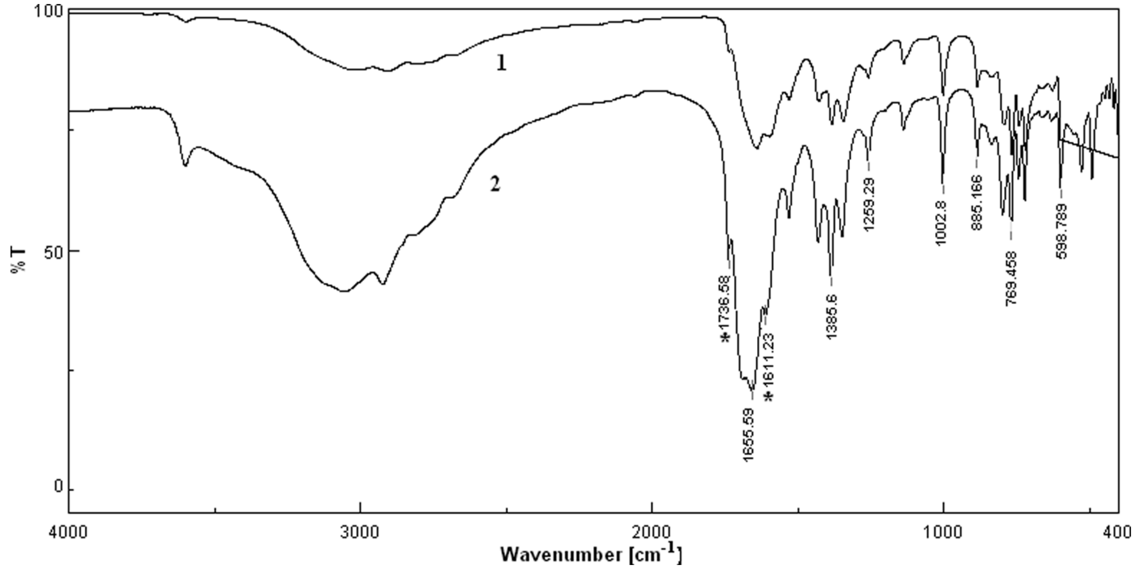


FIGURE 10 Spectra ATR (1) and KPT (2) of Urate Hydrogen Sodium.

the stones was managed by KPT. Table 3 shows that the stones were composed of 11 different components classified according to their detection frequency: WHE; CARB; WED; UAA; AHU; STR; CYS; and rarely SHU, ACCP, BRU, and WHIT, each in less than 1%. The 11 components were distributed in pure stones (9%), binary (38%), ternary (49%), and in quaternary mixtures (4%). These results are in concordance with the similar studies.[1–3,7]

The quantitative study shows that the proportions of species were varying from 0 to 100%: the several determinations performed showed that WHE is the most predominant component in 69% compared with the other components. To know the main component in the stone is of great clinical interest, as it determines the origin of the urolithiasis disorder, except for STR, and ACCP, which are of infectious origin independent of the content.

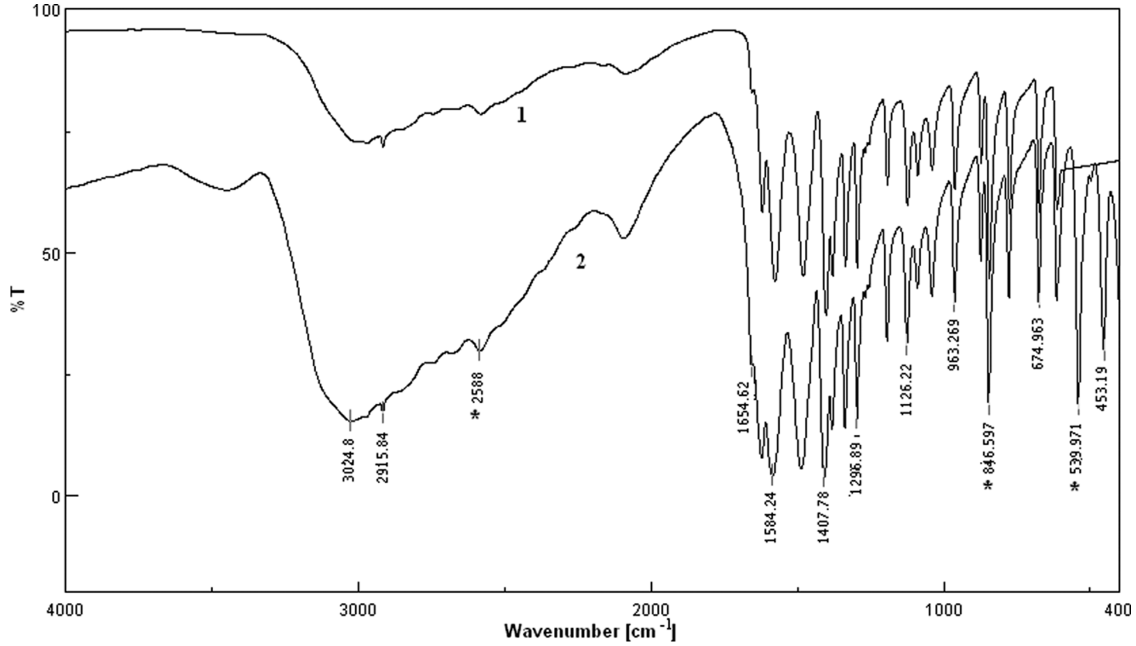


FIGURE 11 Spectra ATR (1) and KPT (2) of Cystine.

TABLE 3 **Stone Components: Frequency of Detection and Predominance**

Component	Frequency of detection (%)	Frequency of predominance (%)
Whewellite	78.9	69
Carbapatite	33.9	3.83
Weddellite	24	9.3
Uric acid anhydrous	19.2	11.5
Ammonium hydrogen urate	7	3.2
Struvite	4.8	1.9
Cystine	1	1
Sodium hydrogen urate	<1	<1
ACCP	<1	<1
Whitlockite	<1	<1
Brushite	<1	<1

4. CONCLUSIONS

The analysis of urinary stones is confirmed as an important investigation for correct treatment and to prevent urolithiasis. The method currently used is the KPT. This technique is more appropriate to determine stones composition, but it is time and sample consuming for sequential identification of all species. To simplify the procedure, we propose optical microscopy for sampling distinct stone zones associated with ATR technique for rapid identification of stone species. The quantification of species in stones of mixed composition is performed finally by KPT. We applied this procedure to study 313 stones. The results show the presence of 11 different stone components with the calcium oxalates as the major chemical group and particularly its monohydrate form (whewellite).

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