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Application of vibrational spectroscopy and XRD analysis for investigation of calcium oxalate kidney stones

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We performed FTIR and XRD analysis to study 78 consecutive urinary stones from 78 patients (58 men and 20 women; age range 18÷66). We have found the highest percentage of stones of calcium oxalate monohydrate - 41%. Half of the stones of calcium oxalate dihydrate are less - 19.2%, and the mixed composition of the studied stones was found in 39.7% of patients: COM + COD - 3.8%; COM + Calcium hydrogen phosphate (CHP) (apatite) - 7.7%; COD + CHP - 2.6%; COD + Uric acid (UA) - 5.1%; COM + UA - 3.8%; COM + COD + CHP - 10.3%; COM + COD + UA - 6.4%. The identification of the components of urinary stones provides useful information in order to understand the cause of their formation and to prevent their recurrence.

Keywords: FTIR, XRD, Kidney stones, Vibrational assignment

INTRODUCTION AND OBJECTIVE

Kidney stones are the products of a pathological bio mineralization process in the urinary system [1,2] and are mostly mixtures of two, three or more components. This disorder is multifactorial in origin and is influenced by the physical–chemical conditions of the urinary system [3]. Determination of the stone type (at both elemental and molecular levels) can help to identify the pathogenesis of kidney stones and to formulate future strategies for the treatment and prevention of this disease in stone-forming patients.

The wet chemical technique is one of the most widely used approaches for stone analysis. However, it can only determine the presence of individual ions and radicals and not differentiate mixtures [4].

A major part of the assessment of the risk of recurrence of urolithiasis in stone-forming patients is the analysis of each eliminated stone, the aim of which is the qualitative differentiation of all stone components and their semi-quantitative and quantitative determination. The wet chemical methods used in our country are sensitive but they can only identify the components of the kidney stone as chemical elements. As a result, the various polymorphs of calcium oxalate, apatite and uric acid salts cannot be separately distinguished. Some authors reported error rate for chemical analysis from 6.5% to 94%, which highlights the extreme inaccuracy of these methods [5].

Until now, only wet chemical methods have been used in Bulgaria for kidney stones analysis. The European Urological Association (EAU) Urolithiasis Guidelines (2017) highlight the aging of the chemical analysis and recommend the use of FT-IR and XRD for kidney stones analysis [6].

Silva *et al.* compared chemical and morphological analyses of kidney stones and reported that unlike the morphological analysis, chemical analysis can detect only Ca and oxalate separately and not differentiate crystalline types. Identifying the crystalline form is very useful for planning therapy, for example, calcium oxalate dihydrate is associated with hypercalciuria, while calcium oxalate monohydrate is more closely related to hyperoxaluria. Thus, these authors suggested using both types of routine analysis for a better understanding of the mechanisms involved in lithogenesis [7]. The use of infrared spectroscopy and / or X-ray diffraction allowed us to find out the crystalline and molecular composition of each eliminated stone, as well as to determine the semi-quantitative and quantitative content of all stone components.

The combined application of IR and Raman spectroscopy technique to the analysis of urinary calculi was reviewed by Carmona *et al.*[8].

The authors documented that some of the characteristic bands are very useful for analytical purposes, further suggesting the adaptability of spectroscopy methods to the routine analysis of renal stones. Paluszkiewicz *et al.* linked the structural composition of renal stones, as determined by FT-IR and FT-Raman spectroscopy, with the elemental composition of the stones determined using protoninduced X-ray emission

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and atomic emission spectroscopy [9]. Based on this study and other published studies, it can be concluded that IR and Raman spectroscopy are the best spectroscopic methods for the identification and quantitative analysis of kidney stones [10]. Both techniques are fast and simple to use, and they only require a small quantity of matter for testing. Raman spectroscopy is used alone or as complementary to IR spectroscopy to achieve the same objective. Both XRD and IR spectroscopy have also been employed as reference techniques for stone analysis, and many studies were subsequently designed to compare the quality of these methods [11,12].

It is also well documented that the analytical results obtained with both the XRD and IR spectroscopy methods are comparable and highly acceptable, consequently, these two techniques can be considered to be reference methods for the analysis of urinary calculi [13]

X-Ray powder diffraction (XRD)

X-ray powder diffraction uses monochromatic X-rays for identifying the constituents of a renal stone based on the unique diffraction pattern produced by a crystalline material. Crystal moieties of structure diffract or reflect penetrated X- rays in particular patterns [4].

Since the 1970s, thermogravimetric analysis (TG or TGA) has been extensively applied for the analysis of kidney stones [14].

This technique is based on the continuous recording of both the temperature and weight loss of the material sampled during a progressive temperature increase to 1,000 °C in an oxygen atmosphere. As each substance has its own specific transformation properties, the starting and ending temperature of the transformation, the amount of the weight change, enthalpy, the nature of the substance and the magnitude of this change indicates the proportion of the elements present [4].

There are different forms in which the same chemical component may crystallize: for example, calcium oxalate may be present in the form of calcium oxalate monohydrate (COM), calcium oxalate dihydrate (COD) or very rarely as calcium oxalate trihydrate (COT). Often, these appear with their mineralogical denominations: whewellite for calcium oxalate monohydrate (Wh); whedellite for calcium oxalate dihydrate (Wd). Uric acid is found in two types of crystallisation: anhydrate (UA) and uric acid dihydrate (UA2). Calcium phosphate is found in multiple forms. In urinary calculi phosphate has many forms: calcium phosphate, calcium hydrogen phosphate dihydrate (brushite), calcium hydrogen phosphate, apatite like carbonate apatite (CAP) and hydroxyapatite (HAP), octacalcium phosphate, a.o. From all the forms in which calcium phosphate can be found, the one which mostly occurs is carbapatite (a carbonate calcium phosphate crystallized in the hexagonal system). Different biochemical conditions can cause several crystallization forms [15]. Consequently, accurate stone analysis must determine not only the molecular species of the calculus, but also the crystalline forms of the chemical constituents.

The aim of our study was to analyze 78 urinary stones with both infrared spectroscopy and XRD and to emphasize the possibility, through these methods, to establish the crystalline and molecular composition of each eliminated stone, as well as to determine semi-quantitatively and quantitatively all stone components.

Materials and methods

We analyzed 78 consecutive urinary stones, from 78 patients (58 men and 20 women; age range 18÷66) hospitalized in the Clinic of Endourology of the Military Medical Academy in Sofia. We used IR-spectroscopy and XRD. FT-IR spectra of all urinary calculi were recorded as potassium bromide (KBr) pellets using a Tensor 37 (Bruker) spectrometer at the Institute of Mineralogy and Crystallography of the Bulgarian Academy of Sciences and the Institute of General and Inorganic Chemistry of the Bulgarian Academy of Sciences. This method requires a small sample of 1-2 mg. In the first stage, the calculi were repeatedly washed with distilled water, and then dried at room temperature for 24 h. Afterwards, a little piece of each calculus was cut with the help of a fine saw; it was examined with a magnifying glass and looked at under a microscope. When the examined section showed a nucleus or other distinctive areas, its parts were studied separately. From the homogeneous stones a piece of approximately 10-20 mg weight was broken and it was powdered in an agate mortar. From it, we weighed a sample of about 2 mg which was mixed with about 200 mg of KBr (Merck-pure for infrared spectroscopy). The mixture was powdered again to obtain practically complete homogenization. This mixture was transferred into an appropriate die and pressed at 10 t/cm^2 to form a transparent pellet, 13 mm in diameter. The pellet, assembled in a holder, was placed in the infrared (IR) beam of the spectrometer. Under this pressure, the potassium bromide mixture is practically vitrified. The spectral region investigated was from 4000 to 400 cm^{-1}; We can measure the spectrum in transmittance or absorbance. The spectrum was printed on a sheet of paper A4 and recorded as an

(ASCI) file. The spectra were measured at a resolution of 2 cm^{-1} and a standard 32 scans. In some of the more complicated cases, up to 100 scans have been used, to improve the signal-noise ratio. The number, position and intensity of absorption bands depend on the molecular composition and crystal structure of the test substance (crystal). The band intensity in the IR spectrum depends on the change in the dipole moment of the connection in the normal vibration process. As much as this change is significant, the band is more intense, if the change is zero, there is no band in the spectrum, i.e it is forbidden for symmetric reasons. In the Raman spectrum band intensity is determined by the change of polarizability over normal coordinate during the normal vibration ðα/ðQ> 0.

In a preliminary stage of our research, in order to find out the type of urolithiasis, we proceeded to the recording of standard IR spectra of the chemically pure compounds found in calculi: organic (oxalic acid and uric acid) and inorganic (phosphates: carbonate apatite, calcium hydrogen phosphate dihydrate, tricalcium phosphate, and carbonates: calcite- calcium carbonates). We used Merck (Germany) and Fluka (Switzerland) compounds. Carbonate apatite (CAP) is a calculus which was chosen out of a multitude of calculi analyzed by FT-IR, by comparing the obtained IR spectra with the one belonging to CAP registered in literature (in the IR atlases) [10,16].

Wide angle X-ray diffraction (WAXD) scans were recorded on a Bruker D8 Advance ECO diffractometer in reflection mode with Ni-filtered Cu Kα radiation over the 2θ range of 1-80 θ with a step of 0.02 θ.

RESULTS AND DISCUSSION

Analysis of the composition of stones eliminated from our patients was performed by using infrared spectroscopy. This made it possible to differentiate between all crystal components in the composition of the stones. For calcium oxalate lithiasis, it is of utmost importance to distinguish the two crystalline forms - calcium oxalate monohydrate and calcium oxalate dihydrate which cannot be achieved with the wet chemical analysis used in our country. We have found the highest percentage of stones of calcium oxalate monohydrate - 41%. Half of the stones of calcium oxalate dihydrate are less - 19.2%, and the mixed composition of the studied stones was found in 39.7% of patients: COM + COD - 3.8%; COM + Calcium hydrogen phosphate (CHP) (apatite) - 7.7%; COD + CHP - 2.6%; COD + uric acid(UA) - 5.1%; COM + UA - 3.8%; COM + COD + CHP - 10.3%; COM + COD + UA - 6.4%.

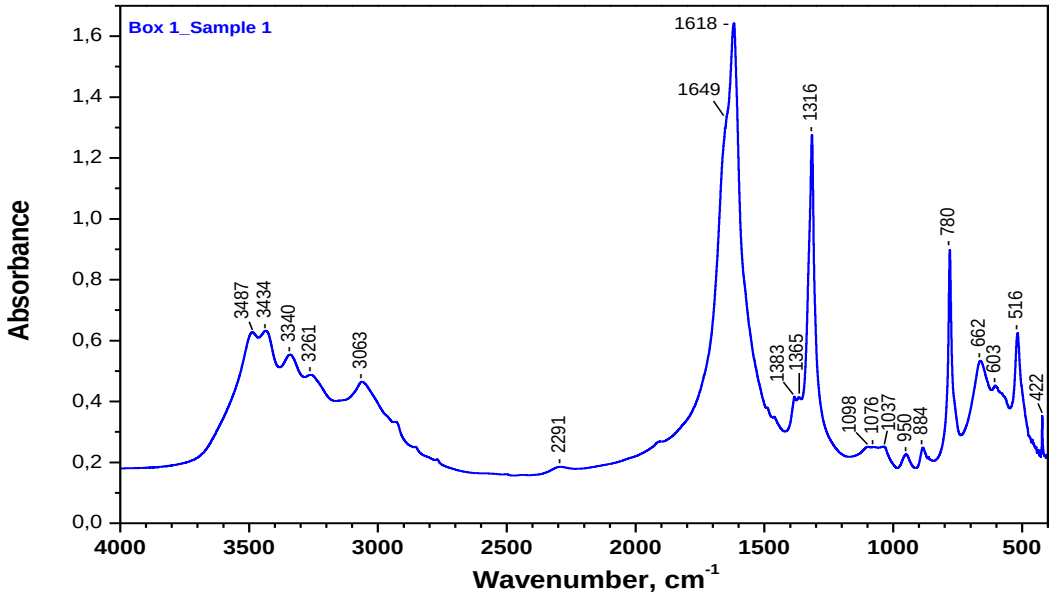

Figure 1. FTIR spectra of calcium oxalate monohydrate

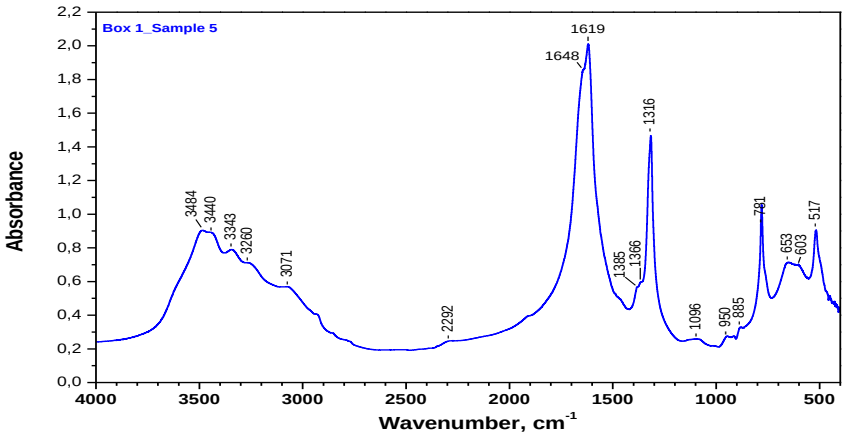

Figure 2. FTIR spectra of calcium oxalate monohydrate and dihydrate

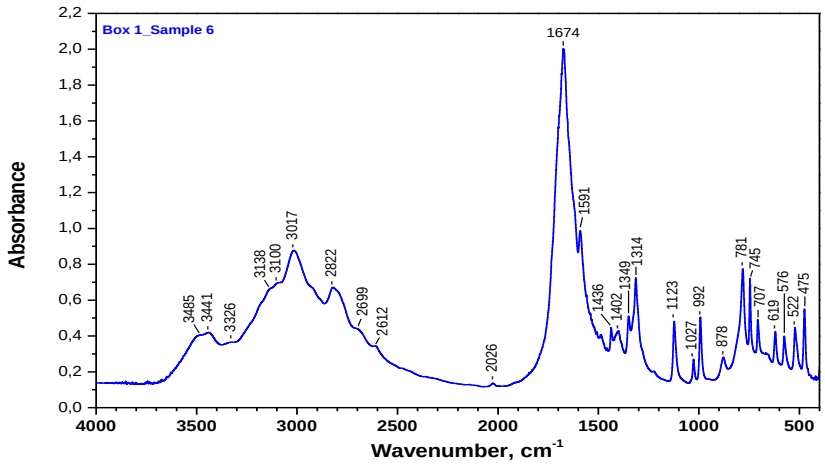

Figure 3. FTIR spectra of calcium oxalate monohydrate and uric acid

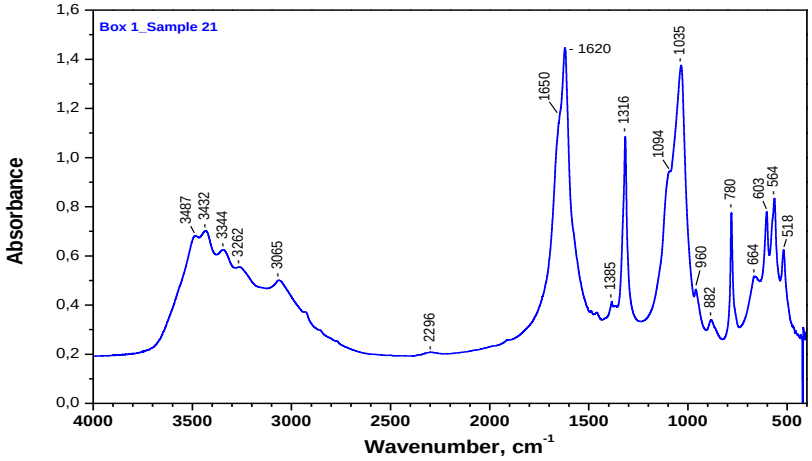

Figure 4. FTIR spectra of calcium oxalate monohydrate and $Ca_5(PO4)_3$ OH

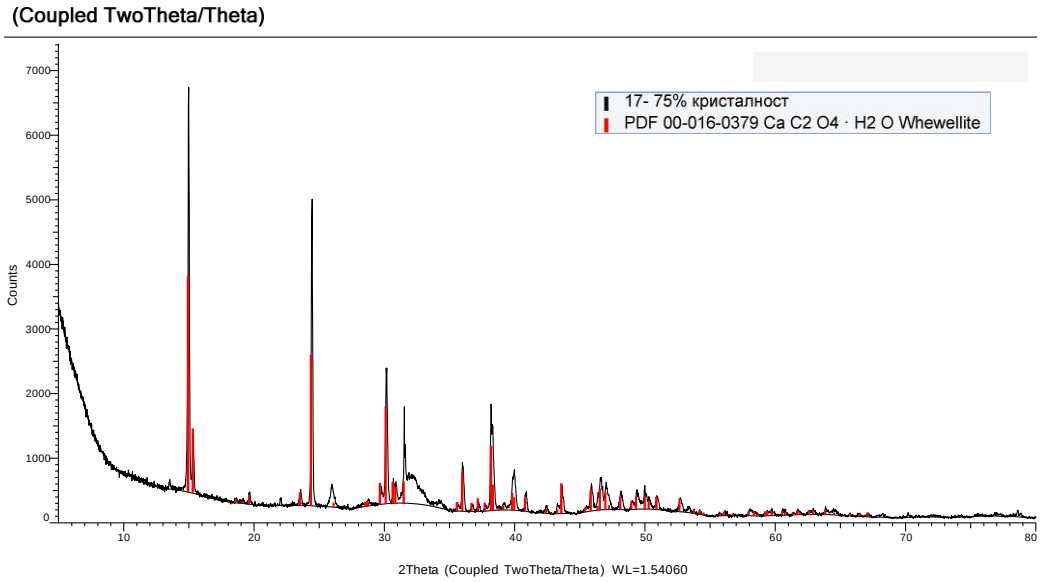

Figure 5. XRD pattern of calcium oxalate monohydrate

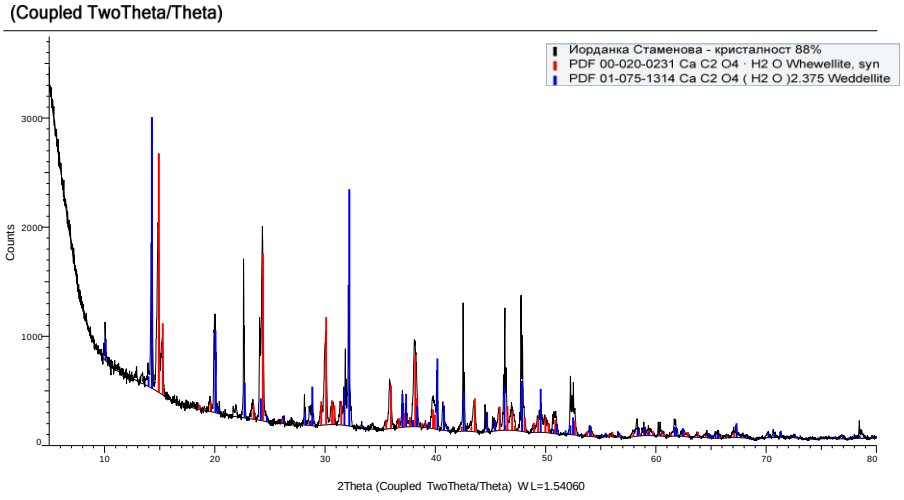

Figure 6. XRD pattern of calcium oxalate monohydrate and dihydrate

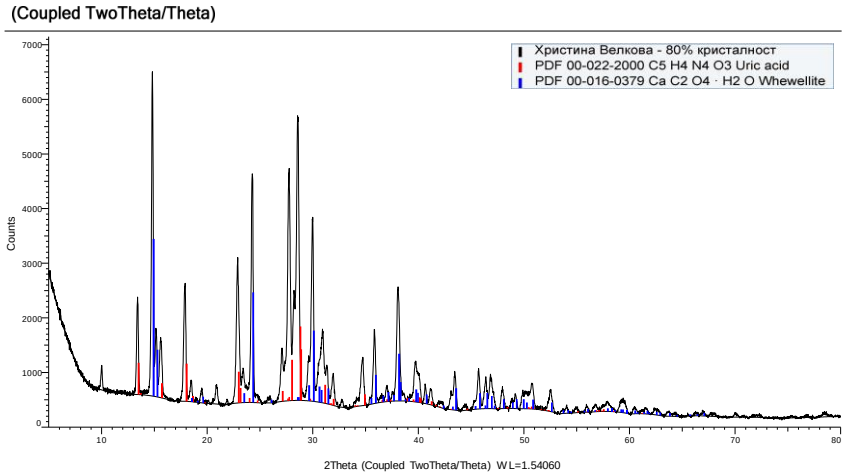

Figure 7. XRD pattern of calcium oxalate monohydrate and uric acid

The spectra of the chemically pure substances were considered as "standard" spectra. By comparing and superposing the calculi spectra with "standard" spectra, we have determined the qualitative and semi-qualitative composition of urinary stones. The intensity of IR bands displays (semi-quantitative) information regarding the degree to which a component's content is found in the calculus (if the compound concentration in the calculus is low, the result will be a specific band of low intensity). The IR absorption of the studied sample has shown the presence of oxalate, uric acid and/or hydroxyapatite groups in the urinary calculi. The reproducibility of the wavenumbers of the peaks may vary in the order of 1-3 cm^{-1}. In some cases, the $\nu^{as}C=O$) has a shoulder at about 1650 cm^{-1}, or the shoulder varies from 1645 to 1650 cm^{-1} in another case [17-19].

The IR band at 1620 cm^{-1} and that at 1316 cm^{-1} (Fig. 1), 1314 cm^{-1} (Fig. 3) of calcium oxalate monohydrate correspond to the symmetric and asymmetric C=O stretching vibrations of coordinated oxalate groups. The IR band at 1618 cm^{-1} and that at 1315 cm^{-1} (Fig. 3), monohydrate calcium oxalate correspond to the symmetric and asymmetric C=O stretchings of coordinated oxalate groups. The strong band at 1620 cm^{-1} appears in a narrow frequency range (1618-1622) cm^{-1}. So this mode is very characteristic. The band at 1316 cm^{-1} is also characteristic – it appears at 1314-1319 cm^{-1} and the band is also very characteristic. For other calculi, the stretching frequencies characteristic to the calcium oxalate were found at 1621 cm^{-1} and 1319 cm^{-1} and were assigned to $\nu^{as}C=O$) and $\nu^{s}C=O$). The degree of hydration of different substances can be given by the presence or absence of some bands from the IR spectrum; therefore, for COM (calcium oxalate monohydrate), this degree is indicated by the two peaks between 850 and 950 cm^{-1} (for COD these two peaks do not appear). In the 3500-3000 cm^{-1} region a broad spectrum appears for COD (calcium oxalate dihydrate), by contrast with the one for COM, which contains four weak but good resolved peaks (Fig. 2). The difference in ν(C=O) of oxalic acid and calcium oxalate is *ca*. 130 cm^{-1}. In literature, the question of the presence of four (O-H) bands is not discussed but only reported as a fact. We conceive that in this case there is a Fermi resonance of both ν^{as}(OH) and ν^{s}(OH) due to the crystal field. Similar phenomena of functional groups of organics, inorganic and complex compounds are described in detail by Kolev and Ivanova [20]. The presence of Fermi resonance and Davydov splitting [21,22] was established by means of polarization infrared spectroscopy in nematic liquid crystals. The band at 3062 cm^{-1} cannot be unambiguously attributed to a certain normal vibration for now. In the literature this band is not commented. For now we cannot adequately assign this band. For the mixed calculi types, these differences cannot be very clearly observed and can be mistaken because of the overlapping of the bands characteristic of different constituents, and for this reason the second derivative has been used [23]. The strong peaks around 1620 cm^{-1} and the weak band at 661 cm^{-1}

could be assigned to the bending and wagging modes of the water molecules.

The bands at 1622 for (COM)/or 1645 (for COD), 1319, 780 and 517 cm^{-1} are due to ν^{a}(C=O), ν^{s}(C=O), δ(O–C=O) and ν(Ca–O), frequencies of the oxalate part [20]. In the literature, the band at 517 cm^{-1} is referred to ν(Ca-O), but this attribution is questionable because this bond has more electro-valent than covalent character, because this is a typical coordination compound like all calcium oxalates. We consider by analyzing the crystalline structure of COM and COD that ν(Ca-O) should be searched at lower wavenumbers, i.e. < 400 cm^{-1} [20]. In previous studies of infrared spectra of carbonyl-containing compounds, the deformation vibrations of the carbonyl groups are in most cases below 400 cm^{-1}, detected with O^{18} labelled carbonyl compounds [24].

IR spectrum of the uric acid is characterized by many bands indicated by the purinic ring absorption bands at 3138, 3020 and 2836 cm^{-1}. The bands are due to N-H stretching vibrations, and also, there are additional bands at 1351, 1124, 1029 and 786 cm^{-1} wave numbers. In the case of urinary calculi, the N-H stretching vibrations appear at 3142, 3026, 2857 cm^{-1} values and there are also IR absorptions at 1353, 1126, 1031 and 788 cm^{-1}, confirming the presence of uric acid in the calculi. Deformation vibrations having frequencies of 567, 603, and 985 cm^{-1}, and stretching vibrations at 1038 cm^{-1} with a shoulder at 1105 cm^{-1} correspond to the apatite mineralogical form of the calcium phosphate. The phosphate ion from CAP/ HAP yields a strong and broad absorption between 1100 and 1000 cm^{-1}. In the best cases there are three well-separated peaks at 1150, 1100 and 1000 cm^{-1}. In some cases, the band at 1150 cm^{-1} is very weak and the only bands remaining are the two bands at 1100 and 1000 cm^{-1}. We can attribute $\nu_3(PO_4)$ and $\nu_4(PO_4)$ to the 3 major peaks of the phosphate anion at 1038, 603 and 567 cm^{-1}. In the work of Popescu *et al*. [23], the band at 567 cm^{-1} is supposedly referred to the vibration lattice mode without giving any explanation for this reference. We think that this referral is too high for lattice vibration, but this assumption has to be confirmed by isotope labelling with O^{18}. For the determination of calcium carbonate we have taken into account absorption values specific to it, at 1415 and 875 cm^{-1}. Carbonate apatite is approximately characterized by the 1422 and 1652 cm^{-1} IR bands. Also, the X-ray diffraction study confirmed the presence of HAP/CAP.

Powder X-ray diffraction, Debye-Scherrer method provides rich information on the structure of the crystallinity grade of many materials from which no suitable sizeable single cristals can be obtained for X-ray determination. The method is widely used today to characterize various materials in modern organic and inorganic material science and also organometallics. The kidney stones, which we studied, are an almost ideal subject for X-ray powder diffraction studies due to their isotropicity. This method gives us information about the formation of crystals in the process of their formation. The magnitude of the crystallites, the degree of crystallinity of the sample, can give in many cases guidance on the different phases of the stone formation process. From this analysis, direct and indirect data on stone formation can be derived. For several years, the Rietveld method has been attempted to obtain the elementary cell parameters. Rietveld refinement is a technique described by Hugo Rietveld for use in the characterisation of crystalline materials. The neutron and X-ray diffraction of powder samples results in a pattern characterised by reflections (peaks in intensity) at certain positions. The height, width and position of these reflections can be used to determine many aspects of the material's structure.

The Rietveld method uses a least squares approach to refine a theoretical line profile until it matches the measured profile. The introduction of this technique was a significant step forward in the diffraction analysis of powder samples as, unlike other techniques at that time, it was able to deal reliably with strongly overlapping reflections.

The method was first implemented in 1967 [25], and reported in 1969 [26] for the diffraction of monochromatic neutrons where the reflection position is reported in terms of the Bragg angle 2θ. In view of the above, we have decided to use the data from this method as a complement to our research. In the case of monocomponent crystals, the match between the results of the two methods is almost perfect, in the case of mixed stones the main components can be identified. If the components are more than three, their determination is of a qualitative nature.

CONCLUSION

The identification of the components of urinary stones provides useful information in order to understand the cause of their formation and to prevent their recurrence. These data are of interest for the clinical guideline in the prophylaxis, therapy and metaphylaxis of urolithiasis. FTIR is also effective in the identification of both the crystalline and amorphous nature of a stone, even when analyzing a small amount of sample. A combination of at least two complementary techniques (in our case FTIR and XRD) would be necessary to obtain enough information on the

morphology, as well as on the molecular and crystalline composition of stones. The data from the XRD method in most cases complement the FTIR spectra, and in some cases the XRD information has its own value.

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