

Figure 5. XRD pattern of calcium oxalate monohydrate

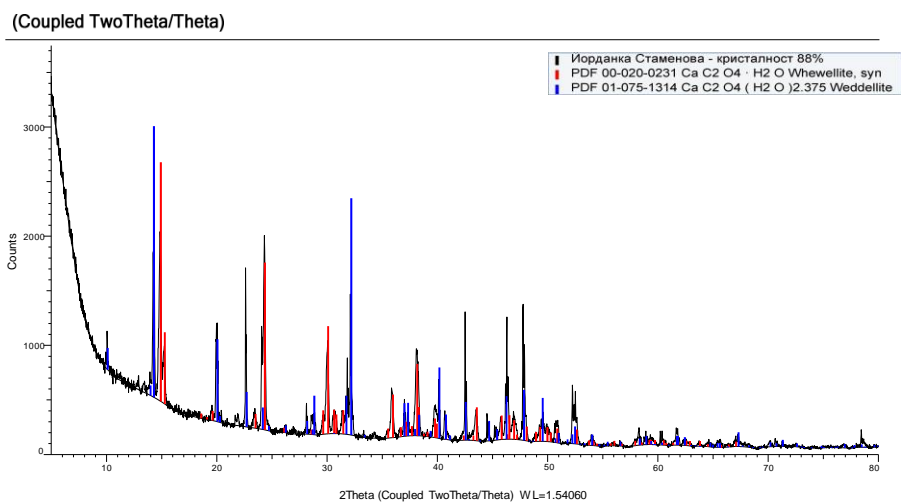


Figure 6. XRD pattern of calcium oxalate monohydrate and dihydrate

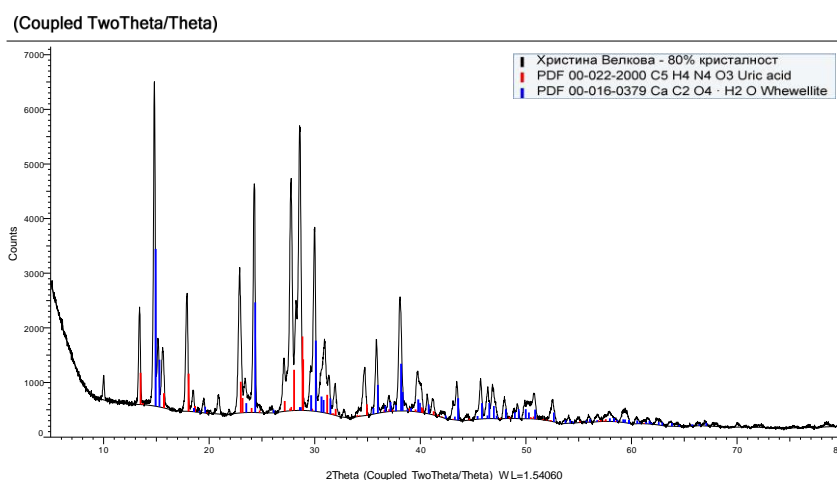


Figure 7. XRD pattern of calcium oxalate monohydrate and uric acid

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 $\nu^{\text{as}}\text{C}=\text{O}$ 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 $\nu^{\text{as}}\text{C}=\text{O}$ and $\nu^{\text{s}}\text{C}=\text{O}$. 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 $\nu(\text{C}=\text{O})$ 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 $\nu^{\text{as}}(\text{OH})$ and $\nu^{\text{s}}(\text{OH})$ 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 $\nu^{\text{a}}(\text{C}=\text{O})$, $\nu^{\text{s}}(\text{C}=\text{O})$, $\delta(\text{O}-\text{C}=\text{O})$ and $\nu(\text{Ca}-\text{O})$, frequencies of the oxalate part [20]. In the literature, the band at 517 cm^{-1} is referred to $\nu(\text{Ca}-\text{O})$, but this attribution is questionable because this bond has more electrovalent 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 $\nu(\text{Ca}-\text{O})$ should be searched at lower wavenumbers, i.e. $< 400 \text{ cm}^{-1}$ [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 $\nu_3(\text{PO}_4)$ and $\nu_4(\text{PO}_4)$ 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 crystals 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

D. Petrova et al.: Vibrational spectroscopy and XRD analysis for investigation of calcium oxalate kidney stones 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.

REFERENCES

1. D. Bazin, M. Daudon, M. C. Combes, C. Rey, *Chem. Rev.*, **112**, 5092 (2012).
2. D. Bazin, M. Daudon, *J. Phys. D: Appl. Phys.*, **45** (2012).
3. M. Daudon, C.A. Bader, *Scan. Microsc.*, **7**, 1081 (1993).
4. G.P. Kasidas, C.T. Samuell, T.B. Weir, *Ann. Clin. Biochem.*, **41**, 91 (2004).
5. I. Singh, *International Urology and Nephrology*, **40**, 595 (2008).
6. C. Türk, EAU Guidelines, ISBN 978-94-92671-01-1.
7. S. F. R. Silva, *Acta Cir. Bras.*, **25**, 444 (2010).
8. P. Carmona, J. Bellanato, E. Escolar, A review, *Biospectroscopy*, **3**, 331 (1997).
9. C. Paluszkiewicz, M. Galka, W. Kwiatek, A. Parczewski, *Biospectroscopy*, **3**, 403 (1997).
10. D. Nguyen Quy, M. Daudon, Infrared and Raman spectra of calculi, Elsevier, Paris, 1997.
11. A. Hesse, H.J. Schneider, E. Hienzsch, *Dtsch. Med. Wochenschr.*, **97**, 1694 (1972).
12. H. J. Schneider, M. Berenyi, A. Hesse, J. Tscharnke, *Int. Urol. Nephrol.*, **5**, 9 (1973).
13. G. Rebentisch, *Scand. J. Clin. Lab. Invest. Suppl.*, **212** (1993).
14. H.P. Lee, D. Leong, C.T. Heng, *Urol. Res.*, **40**, 197 (2012).
15. J. Bellanato, J.E.A. Wickham, A. Colinbuck, Churchill Livingstone (ed.), New York, 1990, p. 45.
16. A. Hesse, G. Sanders, Atlas of infrared spectra for analysis of urinary concrements, Georg Time Verlag, Stuttgart, 1988.
17. C. Bouropoulos, N. Vagenas, P. Klepetsanis, N. Stavropoulos, N. Bouropoulos, *Cryst. Res. Technol.*, **39**, 699 (2004).
18. A.P. Bhatt, P. Paul, *J. Chem. Sci.*, **120(2)**, 267 (2008).
19. K. Nakamoto, Infrared and Raman spectra of inorganic and coordination compounds, John Willey and Sons, New York, 1997.
20. B. Ivanova, T. Kolev. Linearly polarized IR spectroscopy. Theory and Applications for Structural analysis. 2011, CRC Press, Taylor & Francis Group, Boca Raton, US, 2011.
21. A.S. Davydov, Theory of Molecular Excitons, McGraw Hill, New York, 1962.
22. A.S. Davydov, Theory of Molecular Excitons, Nauka, Moscow, 1968.
23. S.G. Popescu, I. Ionescu, R. Grecu, A. Preda, *Revista Română de Medicină de Laborator*, **18**, 4, (2010).
24. T. Kolev, *J. Mol. Struct.*, **349**, 381 (1995).
25. A. Heat, O.F. William, E. David, L. van Eijck, *J. Appl. Cryst.*, **49**, 1394 (2016).
26. H.M. Rietveld, *J. Appl. Cryst.*, **2**, 65 (1969).