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Abstract. Results of the structural analysis of urinary sediments by means of infrared spectral microscopy are presented. The results are in good agreement with the results of standard optical microscopy in the case of single-component and crystalline urinary sediments. It is found that for noncrystalline or multicomponent sediments, the suggested spectroscopic method is superior to optical microscopy. The chemical structure of sediments of any molecular origin can be elucidated by this spectroscopic method. The method is sensitive enough to identify solid particles of drugs present in urine. Sulfamethoxazole and traces of other medicines are revealed in this study among the other sediments. We also show that a rather good correlation exists between the type of urinary sediments and the renal stones removed from the same patient. Spectroscopic studies of urinary stones and corresponding sediments from 76 patients suffering from renal stone disease reveal that in 73% of cases such correlation exists. This finding is a strong argument for the use of infrared spectral microscopy to prevent kidney stone disease because stones can be found in an early stage of formation by using the nonintrusive spectroscopic investigation of urinary sediments. Some medical recommendations concerning the overdosing of certain pharmaceuticals can also be derived from the spectroscopic studies of urinary sediments.
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Keywords: urinary sediments; urinary stones; Fourier transform infrared microscopy; urolithiasis.

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1 Introduction

Kidney stone disease, or urolithiasis, is a heterogeneous group of various pathological-metabolic cascades leading to the development of stones of different chemical nature in the urinary tract. In industrialized European countries, the prevalence of kidney stones continually increased throughout the twentieth century.[1] There are considerable differences in the prevalence and composition of stones among countries and also within the same country.[2] According to some epidemiological studies, kidney stone composition has changed from predominantly urate and phosphate to calcium oxalate, and now approximately 80% of stones are composed of calcium oxalate monohydrate (COM) and calcium phosphate (CaP), 10% are struvite (magnesium ammonium phosphate produced during infection with bacteria that possess the enzyme urease), 9% are uric acid (UA), and the remaining 1% are composed of cystine or ammonium acid urate or are diagnosed as drug-related stones.[3] Age has a prominent role in the etiopathogenesis of kidney stones in both genders. Some authors point to the increase in urate stones with age in both genders and the preponderance of phosphate stones in women.[4]

The likelihood of developing kidney stones in one's lifetime is about 13% for men and 7% for women.[5] The possibility of having another stone-related event after initial stone removal is unacceptably high—approximately 50% during lifetime and shows the lack of appropriately targeted prevention.[6] Furthermore, it is well known that kidney stone disease increased in prevalence in adults aged 20 to 74 over the past 20 years, a possible explanation being the shift in dietary habits of people in developed and developing countries.[7] Current guidelines of taking in more fluids and eating a multicomponent diet can, however, only target general pathways in stone-formation processes and do not meet the standards for individual-based health care that directs therapy toward the underlying causes of specific stone formation.[8] Primary prevention strategies for stone disease have not been sufficiently evaluated, but if supplemented by the metabolic work-up of individual kidney stones and urinary sediments could be beneficial and cost-effective.[9] Information about the chemical structure of kidney stones is of great importance for the treatment and prevention of uronephrolithiasis. The usefulness of such information was already recognized in the early 1950s.[10]

In our previous studies, we have shown the importance of Fourier transform infrared spectroscopy (FTIR) for investigating the chemical composition of kidney stones.[11] Our results confirmed that FTIR spectroscopy is an effective experimental tool to determine the chemical components of kidney stones. The method is sensitive to the organic and inorganic components

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of the stones. Even a very small amount of material can be easily detected by the FTIR method. By using FTIR spectroscopy, we showed that outward similarities or differences in kidney stones are not sufficient argument for attributing them to certain types.[11]

A key condition for uronephrolithiasis to start is urine oversaturation with some specific chemical components, which leads to crystal growth and aggregation. Early discovery together with the identification of the exact chemical composition of urinary crystals could be crucial for taking appropriate preventive measures that inhibit kidney stone formation or growth processes. However, the presence or absence of urinary crystals is rarely associated with some specific symptoms of kidney stone disease until an actual stone forms in the urinary tract.[12] Optical microscopy is now the main method used to test urinary sediments in order to diagnose crystalluria and to identify regularly shaped (e.g., crystalline) urinary deposits.[13] To determine the chemical structure of amorphous sediments or crystal clusters, however, optical microscopy cannot be considered a reliable method.

The first results of the application of FTIR microscopy (mFTIR) for urinary crystals of kidney stone formers were published in 1991 by Daudon et al.[14] In later research, the same group extended studies of urinary crystals combining FTIR with the potassium bromide (KBr) pellet technique instead using only FTIR microscopy. Combining spectral results for urinary sediments and kidney stones obtained with the KBr pellet technique with the results of optical microscopy of the sediments, the researchers found 97.3% correlation between stone type and crystals type in urine sediment.[15] These studies were restricted because urinary crystals can be investigated by KBr pellet technique only when a significant amount of crystals precipitate. In 2011, the results of crystalluria investigation by mFTIR method for patients with a wide range of renal diseases and only for urinary crystals that cannot be identified by optical microscopy were published by the same team.[16] However, there is a lack of combined studies of "typical" and "atypical" urinary sediments by means of mFTIR and urinary stones by means of the KBr pellet technique.

In this paper, we examine the chemical structure of urinary sediments (using FTIR microspectroscopy) from patients with kidney stone disease and the chemical structure of kidney stones (using FTIR spectroscopy with the KBr pellet technique) removed from the same patients. The aim of the study is to find the correlation between the chemical composition of urinary sediments and kidney stones.

2 Materials and Methods

An experimental setup consisting of a Vertex 70 FTIR spectrometer and Hyperion 3000 infrared (IR) microscope (Bruker Optik GmbH, Ettlingen, Germany) was employed to record the IR absorption spectra of the samples. The spectra of powdered kidney stones embedded in a KBr pellet were recorded using transmission mode of the FTIR spectrophotometer, and the IR absorption spectra of urinary sediments were recorded by the IR microscope and the FTIR spectrometer. The microscope was used either in visible light or in infrared radiation modes. A CCD camera in visible light mode helps to find specific places of interest of a sample. For the optical investigation of the morphology of urine sediments, different objectives with either 15× or 4× times magnification were used depending on the size of the deposits. With the 15× magnification objective the size of the single deposit possible to resolve was around 5 μm. In the case of 4× magnification the size is around 20 μm. The 4× objective was only used when the area of single deposit was too big to fit in the single visible light overview image taken with 15× (more than 180×180 μm).

The infrared mode of the microscope was used to obtain IR absorption spectra of the mid-infrared spectral region. Spectral resolution of the infrared spectra was 4 cm^{-1}. One hundred twenty-eight spectra were accumulated and averaged to produce one resultant spectrum. IR radiation was collected with single-channel semiconductor mercury-cadmium-telluride (MCT) detector cooled by liquid nitrogen to 77 K.

We analyzed urine samples and urinary stones of 76 patients suffering from kidney stone disease and hospitalized at Vilnius University Hospital Santariskiu Clinics.

Urine samples were collected just before the kidney stone removal surgery and were centrifuged leaving mostly urea and urine sediments in the samples. Then, after being placed on a filter (Whatman 542), the urine was left for 24 h to dry. Then the isolated crystals of the sediments were collected from the surface of the filter and transferred to the transparent for IR radiation window (made of CaF_2 or ZnSe) to analyze them. Crystals that were big enough (about 50 to 100 μm) were transferred using the small needle.

The spectra of the sediments were recorded using IR transmission mode of the microscope. Most of sediments, crystals, or crystals clusters were too thick to record appropriate transmission spectra for qualitative analysis. For this reason, crystals were squeezed between two IR transparent optical windows. In such a way the sediments were crushed and suitable sample thickness (about 10 to 20 μm) was achieved. Then one of the optical windows was removed for IR radiation to reach the sample directly. Optical surface damage on CaF_2 optical windows caused by crushing the urinary crystals between them was minor. Although it was more pronounced in the case of ZnSe optical windows, the damages were small enough not to cause essential scattering impact to the quality of acquired spectra. In our experiments, the spectra were primary acquired placing the crystals on CaF_2 optical windows. If the information provided by given spectral region was not enough, the crushed deposits were displaced on ZnSe optical windows. In this way avoiding the damage for the optical windows was most effective; nevertheless, the single experiment was more time consuming.

In order to maintain good signal-to-noise ratio of the spectral bands, the standard imaging area (100×100 μm) of the microscope was reduced to dimensions of the sediments (down to 15×15 μm) by means of the aperture of the microscope. This reduction is needed in order to reduce spectral noise caused by the infrared radiation that does not interact with the sample. For micro-scale sediments, however, the low absorbance of radiation was the main reason to limit the minimal size of single crystals, which could be properly identified by our microspectrometer. We chose filters with 2.7 μm particle retention to separate the crystals suitable for investigation.

Samples of the kidney stones for the FTIR method were prepared using the potassium bromide (KBr) pellet technique. Each kidney stone was ground using an agate mortar. Approximately 2 mg of ground kidney stone was mixed with 200 mg of KBr and compressed into a pellet by using a manually operated hydraulic press "Specac." This pellet was then attached to special holder and placed into the sample compartment of the spectrometer to register the spectrum.

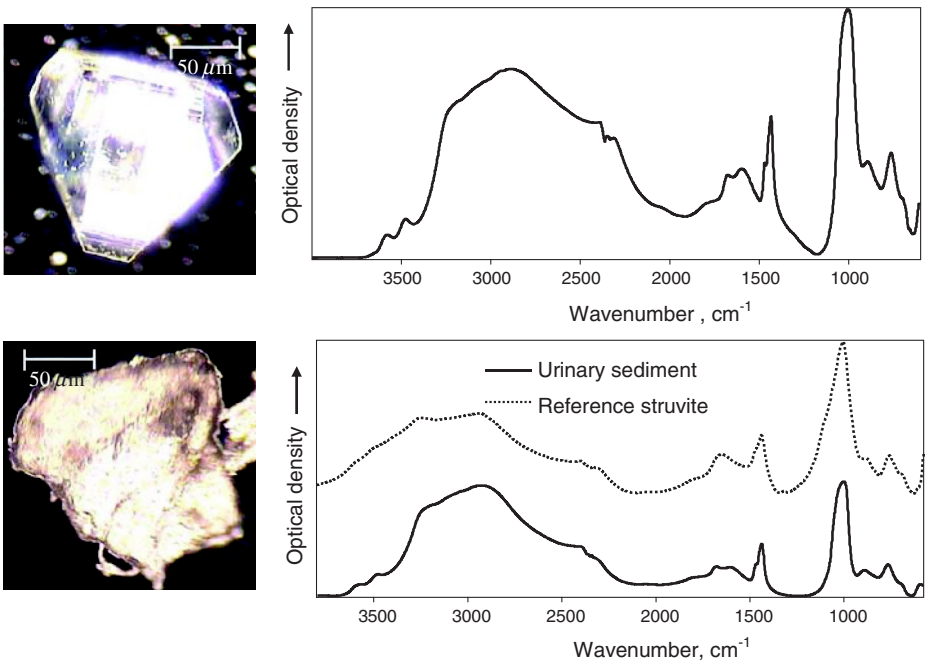

Fig. 1 Struvite crystals having different morphological shape and their IR spectra both corresponding to struvite.

To identify the chemical composition of urinary sediments and kidney stones, every IR spectrum measured was compared with the corresponding reference pure chemical compound spectrum.

3 Results and Discussion

Before the investigation of the urine samples of the kidney stone formers, the urine of healthy individuals was examined for the reference purposes. No crystals were found in any of the urine samples of the healthy people investigated. There are two possible conclusions for this: first, that the urine of a healthy person is not supersaturated with any stone-forming substances; second, that the crystals were too small to stay on the filter when filtered. Neither reason influences the risk of urolithiasis at the present period of the life of the person.[17]

Urine of urinary stones formers was then examined to find out whether the presence of crystals in urine could indicate kidney stone disease. Urine samples of 76 patients were investigated, and crystals, crystal clusters, or organic clusters were found in all samples. The main kidney-stone-forming materials were found by FTIR method either as single crystals or as components of crystal clusters: COM, calcium oxalate dihydrate (COD), UA anhydrous (UAA), UA dihydrate (UAD), ammonium acid urate (AAU), hydroxyapatite (HAP), brushite, and struvite.

When observed in the visible light mode of the infrared microscope, urinary sediments were found either as crystals having a regular morphological structure, amorphous sediments, or disordered crystal clusters. Optical investigations combined with the identification of the chemical composition of the sediments revealed that the morphological structure of urinary crystals does not rigorously determine their chemical structure. As an example, the optical images of one crystal from patient's urine having well defined crystalline structure and another with irregular shape from the other patient's urine are presented in Fig. 1. Both sediments are built from struvite—their spectra resemble infrared absorption spectrum of pure struvite. Single crystals having a chemical composition that could not be defined by the optical investigation of their morphology; for example, brushite or calcium oxalate (see Fig. 2) are easily identified by analyzing their infrared absorption spectra and comparing them with corresponding pure component spectra.

Crystal clusters containing more than one chemical substance indicate urine oversaturation with several urinary-stone-forming materials that could form multicomponent urinary stones. However, optical investigation of such crystals does not reveal whether it is a single crystal or several different chemical components that have unified into one cluster. Figure 3 shows urine sediments composed of calcium oxalate with HAP and calcium oxalate with UA. The IR spectra of the cluster shown in Fig. 3(a) have characteristic IR absorption bands at 1620 and 1315 cm^{-1}, indicating COM due to antisymmetric and symmetric C-O stretching, respectively.[18] Absorption bands at 1458 and 1420 cm^{-1} caused by of CO_3^{2-} group vibrations and intensive absorption at 1036 cm^{-1}, indicate HAP.[19] The mixture of COM and UAA or urinary sediment containing only UAA can be distinguished by a single absorption band at 1320 cm^{-1} in the case of the mixture and two characteristic bands at 1347 and 1301 cm^{-1} for pure UAA urinary sediment. The IR spectrum of the cluster in Fig. 3(b) depicts sediment consisting of the mixture of calcium oxalate and UA.

Four of 76 investigated samples contained urinary crystals that were composed of more than two different materials. Figure 4 shows a urinary crystal that was composed of COM, AAU, and HAP. The absorption bands of COM are identified at 1620, 1315, 782, and 667 cm^{-1} while 1436, 1388, 1007, and 884 cm^{-1} are spectral bands that belong to AAU, and they are reasoned by vibrations of purine rings. Absorption at 1036 cm^{-1} indicating stretching vibration of PO_4^{3-} group[20] is characteristic for HAP.

We were able to distinguish different hydrates of calcium oxalate (monohydrate and dihydrate form) and various urates

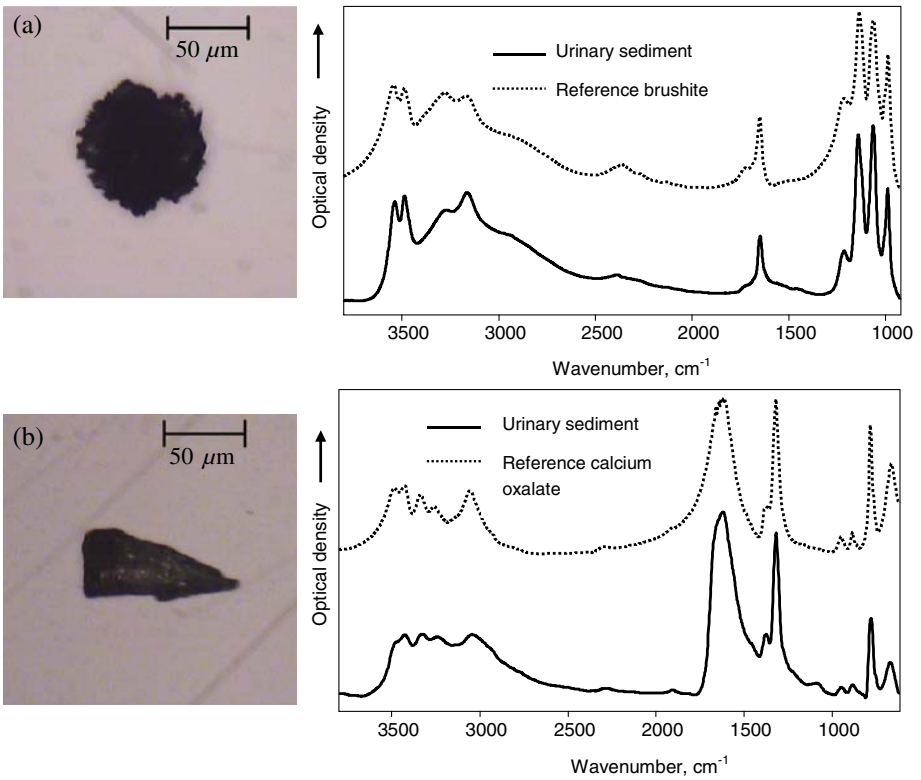

Fig. 2 Optical images corresponding with brushite (a) and calcium oxalate (b) and their IR spectra together with the IR spectra of pure chemical components.

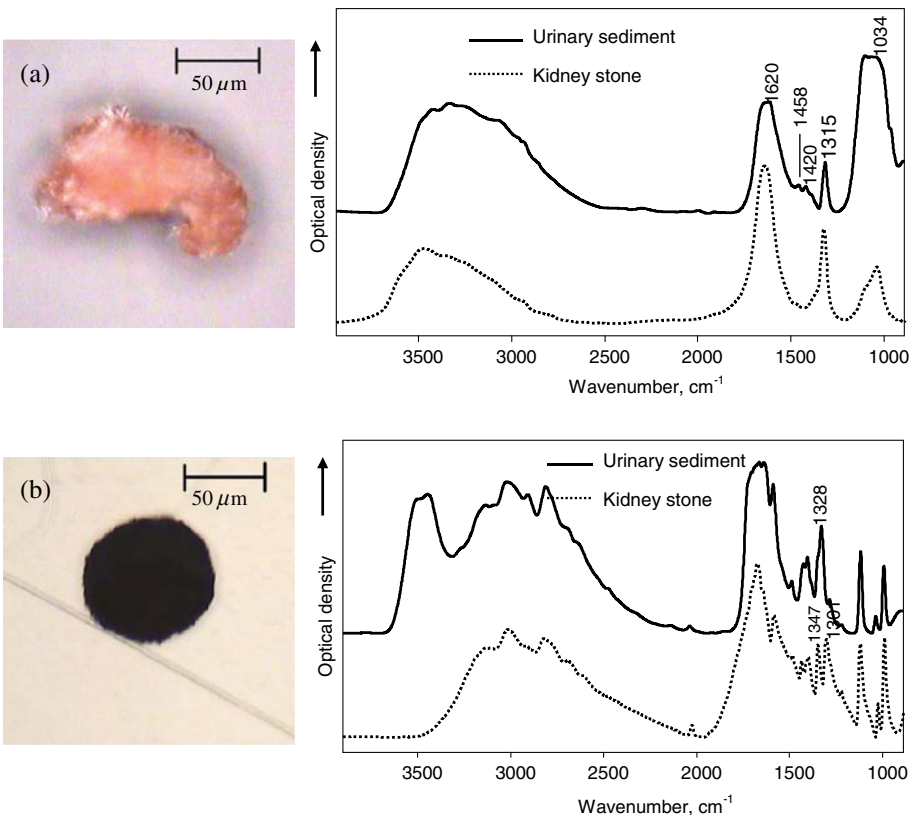

Fig. 3 Optical images of a cluster of calcium oxalate and hydroxyapatite (a) and calcium oxalate and uric acid (b) and their IR spectra compared with the IR spectra of the kidney stones of the same patients.

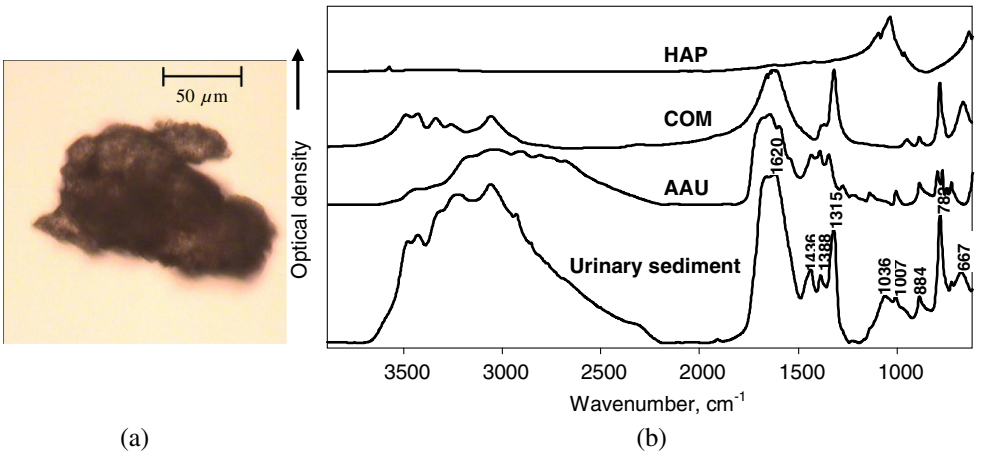

Fig. 4 Optical image of urinary sediment (a) and corresponding IR spectrum (b) where characteristic spectral bands are assigned to COM, AAU, and HAP.

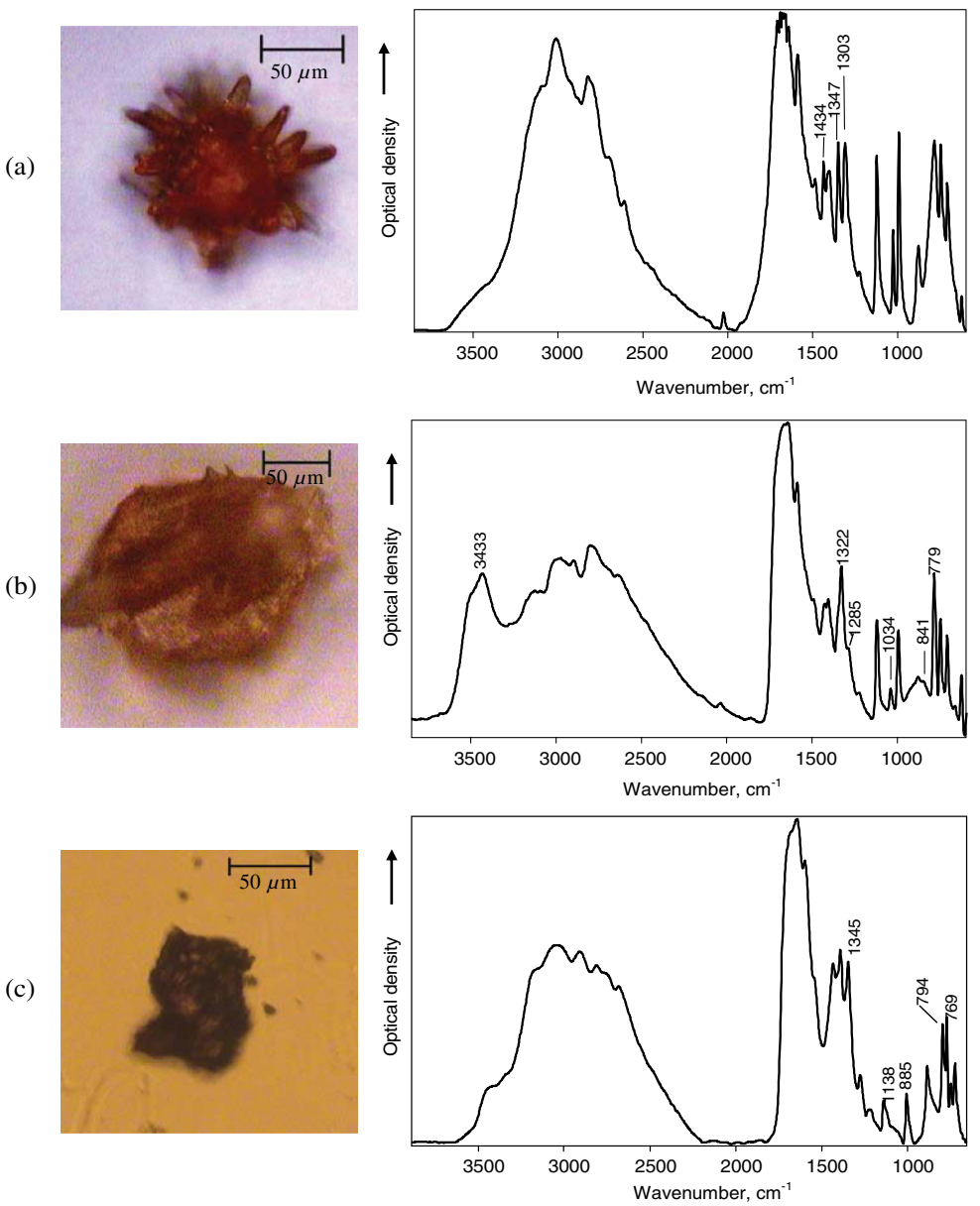

Fig. 5 Optical images of uric acid anhydrous, (a) uric acid dihydrate, (b) and ammonium acid urate (c) urinary sediments and their IR spectra.

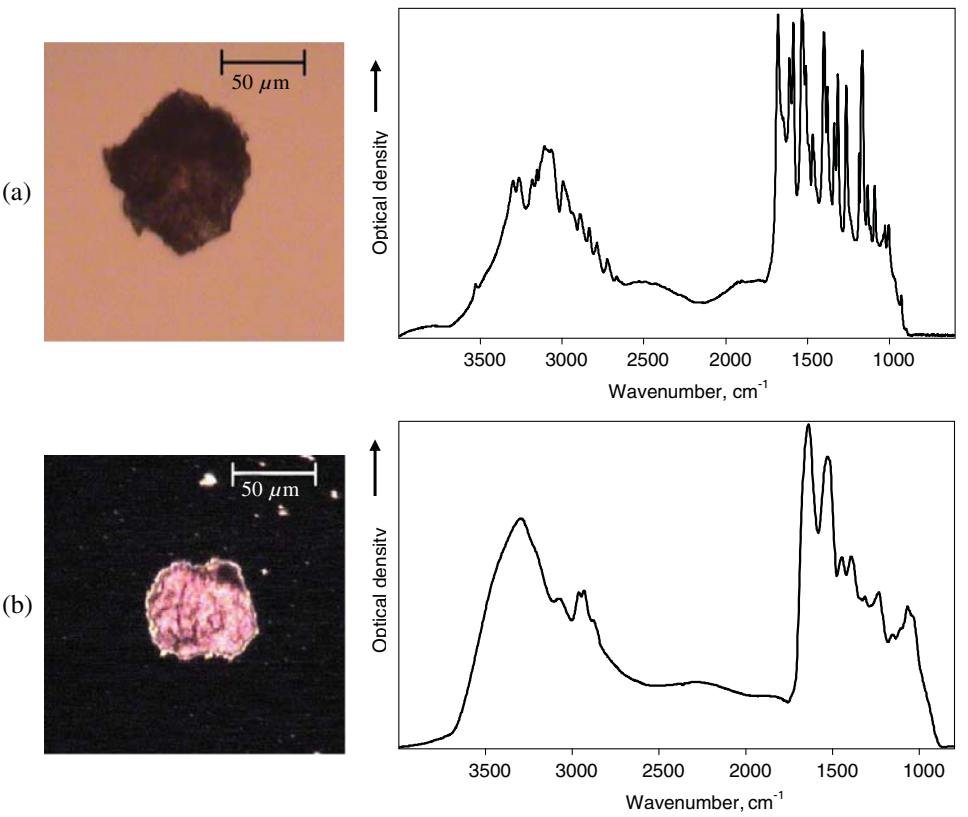

Fig. 6 Optical images of *N*-acetylsulfametoxazole (a) and organic sediment (b) and corresponding IR spectra.

(UAA, UAD and AAU) in urine sediments. Such information is important for the more detailed modeling of possible urinary stone formation mechanisms and the suitable choice of preventive actions. IR spectra and wavenumbers of characteristic spectral bands of different urinary sediments that were composed of various urates can be seen in Fig. 5. Again, optical images of corresponding urine deposits provide no relevant information.

Infrared microspectroscopy is informative enough to identify the chemical composition of some atypical urinary deposits that do not form urinary stones but can influence the formation processes. Figure 6(a) shows the corresponding IR spectrum of a drug metabolite crystal called N-acetylsulfamethoxazole. Drug-induced urinary stones are not very common. They are often unexpected, and preventive measures cannot be undertaken.[21] Thus early discovery of crystalline drug metabolites may allow more efficient prevention and could provide relevant information about their formation. Organic sediments can also be easily determined as shown in Fig. 6(b).

In 73% of the cases of the 76 patients investigated, the one's urinary sediments and urinary stone had at least one urinary-stone-forming chemical compound in common. Exactly the same composition was found in 41% of those cases. We divided urinary stones in three categories: (1) oxalate stones (consisting of pure calcium oxalate or calcium oxalate with impurities of CaPs); (2) uratic stones (consisting of pure UA or mixed uratic stones); (3) phosphatic stones (consisting of struvite or brushite). Oxalate stones were the most common, 67% of all stones; 28% of kidney stones were uratic, and the remaining stones were phosphatic. As shown in Fig. 7, in only 27% of the cases did the chemical composition of oxalate urinary stones

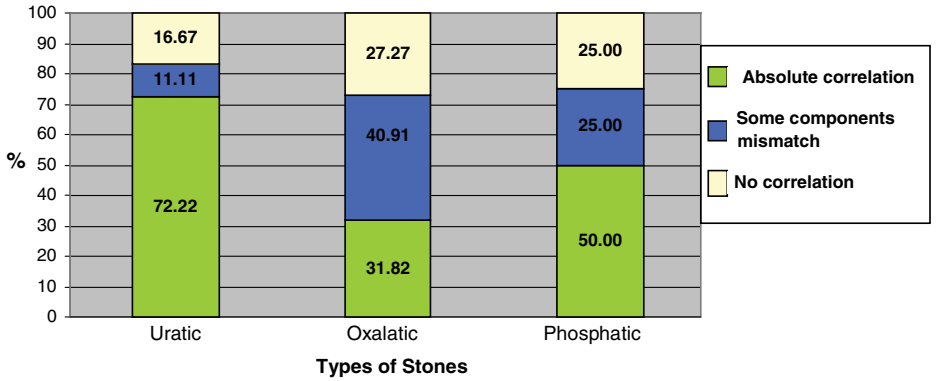

Fig. 7 Correlation of the chemical composition of urinary sediments and kidney stones according to different types of urinary stones.

and corresponding urine sediments exhibit absolute correlation. Organic sediments were mostly found instead. It was already shown by some authors that organic matrix has a lot of influence on oxalatic stones formation processes.[22] Also, other types of CaPs than those that were in oxalate urinary stones were sometimes discovered as sediments. This is in the agreement with the proposed theory stating that phosphates form via reactions from other phosphatic precursors in urine when different pH or ion concentration conditions are met.[23]

The situation is different with uratic stones. Absolute correlation of chemical composition with urinary deposits was observed for 71% of uratic kidney stone patients. This suggests that uratic urinary stones could be greatly suspected when urine is oversaturated with UA.

Phosphatic kidney stones are rare, and we found just few of them. In 75% of cases, the correlation was determined when compared with the chemical structure of urinary sediments. More cases of phospatic kidney stones and corresponding urinary sediments should, however, be investigated for the results to be statistically reliable.

4 Conclusions

We showed that FTIR spectroscopy is an effective and sensitive method to determine the chemical components of kidney stones. Using the mFTIR method in this study, we demonstrated that this technique is informative, reliable, and easy to use to define the chemical composition of urinary crystals no matter their morphological structure. Moreover, mFTIR is especially useful for the investigation of unusual urinary crystals and clusters of crystals containing more than one chemical element, which indicate urine oversaturation with several urinary-stone-forming substances. The correlation between the chemical composition of kidney stones and urinary sediments suggests that the examination of sediments by mFTIR can be considered a relevant technique for the early identification of kidney stones and determination of appropriate action to prevent their formation. In contrast with optical microscopy, infrared spectral microscopy does not rely on the skills of laboratory personnel; thus there is no significant possibility of misinterpreting the chemical compounds of urinary crystals. Combining optical investigation with the infrared spectral investigation of the chemical composition of sediment, we found that the optical view of the morphological structure of urinary crystals does not strictly determine their chemical structure. Optical images of corresponding urine deposits provide no relevant chemical information, though this information is important for the further investigation of possible mechanisms of kidney stone formation and for more effective prevention of urolithiasis. To know the chemical composition of urinary sediments is important to identify risk factors for recurrent stone events. This could reduce the negative effects that kidney stone disease has on an individual patient's quality of life and on the public health system in general.

Acknowledgments

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