Characterization of Kidney Stones type using Raman Spectroscopy and Electron Microscopy

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Abstract— Raman spectroscopy (RS) is an excellent photonic measurement technique that can be extremely useful for characterizing kidney stones. Using RS, we can obtain precise information about the chemical composition and microstructure of the crystals that comprise kidney stones. Therefore, this non-invasive technique makes identifying different types of stones possible. Additionally, Raman spectroscopy supported by electron microscopy (SEM) imaging enables us to relate a given kind of crystal to its morphology. This paper presents the results of ex situ analysis of real samples of an unknown type of human kidney stone using RS and SEM techniques, which led to attempts to identify the stone.

Kidney stones are a global health issue that affects up to 20% of people worldwide [1-2]. The formation of deposits just a few millimeters in size can cause physical pain and lead to chronic kidney disease. Those at risk are mainly elderly individuals, though the problem also. affects younger people and even children. The type of kidney stones a patient has is examined to minimize the chance of recurrence and prevent new stones from forming. One effective method used in laboratories to assess their composition is Raman spectroscopy (RS) [3-5]. RS has several advantages that make it a promising diagnostic technique for *in-situ* application in medical operating rooms. First, RS does not require special sample preparation and is insensitive to the variable water content of the sample. Using fiber optic solutions allows real-time imaging of the laser ablation process during surgery [6].

This paper presents an attempt to identify an unknown type of renal stone using the RS technique supported by imaging performed by electron microscopy (SEM). The images were taken using an INSPECT S50 (FEI, Hillsboro, OR, USA). The study aimed to compare the surface morphology with the spectral measurements obtained by the RS technique. Spectral analysis of renal stone specimens was performed using the RS technique with excitation by 633 nm and 532 nm laser beams on an NTEGRA Spectra system (NT-MTD, Eindhoven, the Netherlands). Different wavelengths of the excitation beam were used to highlight the limitations of the RS technique in analyzing these kidney stone samples.

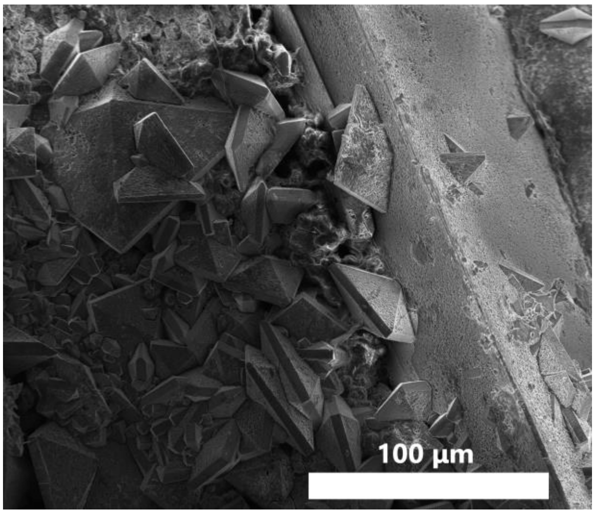

Fig. 1. SEM image of the kidney stone surface, sample no. 1. HV = 5kV.

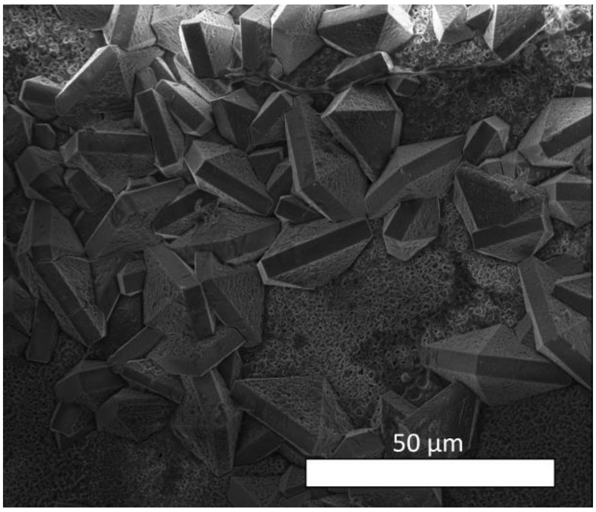

Fig. 2. SEM image (zoom) kidney stone surface, sample no. 1. HV = 5kV.

The preparation of the samples, the composition of which was unknown, began with cleaning them of potential contaminants, such as residual blood or tissue. Then, the stone samples were placed in separate tubes filled with distilled water. The samples were left in this state for 24 hours and then underwent a drying process (120 hours at room temperature). It was vacuum-coated

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with a thin layer of gold to prepare one of the samples for effective imaging and SEM analysis with minimization of the charging effect on the sample.

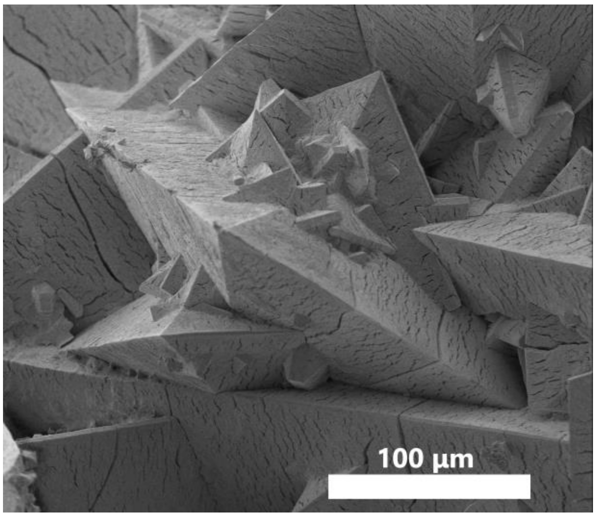

Fig. 3. SEM image of the kidney stone surface, sample no. 2 covered with Au, HV = 5 kV.

The first stage of the study involved examining the surface morphology using SEM. This examination was performed at various locations on the specimens, with different electron beam energies and magnifications. Figures 1 and 2 show the surface layer of the deposit. Bipyramidal shapes were observed, which were identified as calcium oxalate dihydrate [7-10]. These shapes densely covered the surface, forming structures stacked on top of each other. This indicates that, if it was not expelled, the stone would continue to increase in size.

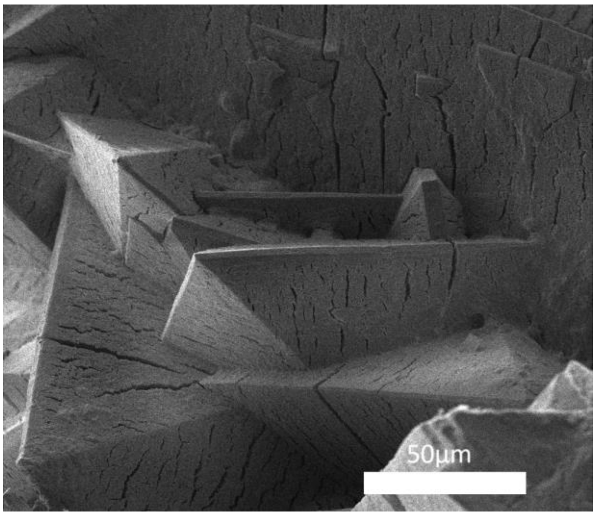

Fig. 4. SEM image (zoom) kidney stone surface, sample no. 2 covered Au., HV = 10 kV.

Calcium oxalate dihydrate is a common component of kidney stones. Compared to calcium oxalate monohydrate, it indicates a higher calcium content and a lower urinary oxalate level. This type of stone formation is caused by insufficient fluid intake and increased oxalate and calcium concentrations [11].

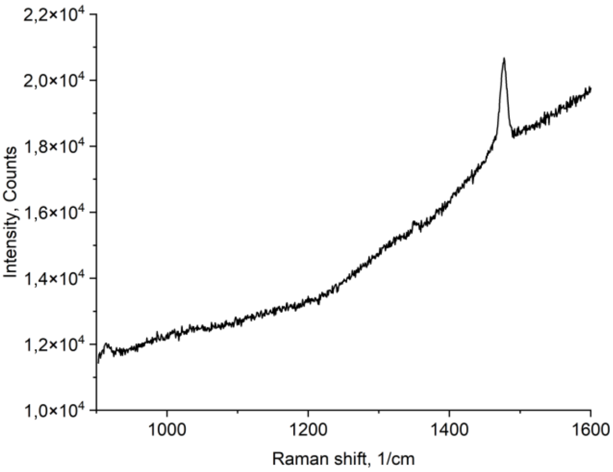

Fig. 5. Raman spectra, excitation 532 nm, sample no. 1, spectra raw data.

The second test stone was covered with a thin layer of gold to minimize the charging effect of the test sample, which is undesirable during SEM analysis (Fig. 3, Fig. 4). For higher electron energy (HV acceleration voltage), the effect of better imaging of the sample was obtained, which improved the identification of morphological details of the stone structure and thus its type. SEM studies show that the stone is calcium oxalate dihydrate (Weddellite) [7-10]. No other characteristic structures indicative of other types of stone were observed in the analyzed morphology.

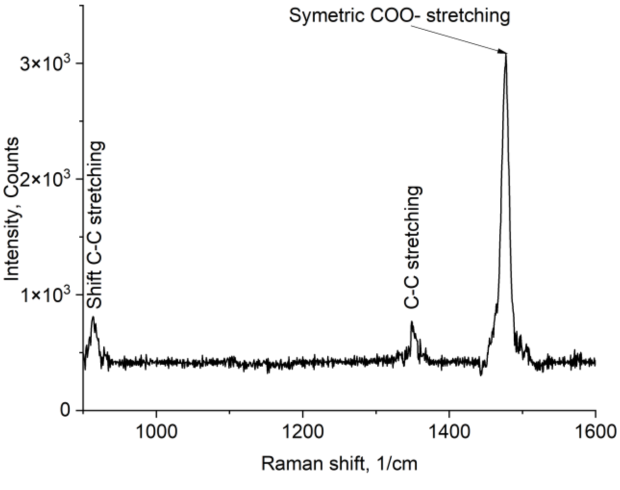

Fig. 6. Raman spectra, excitation 532 nm, sample no. 1, processed spectra data.

The second stage of the research involved using photonic methods to determine and confirm the composition of the stone based on its scattering spectrum. To this end, the RS technique was employed. First, tests were conducted using laser beam excitation with a wavelength of 532 nm. This type of excitation was chosen based on the initial identification of the stone using the SEM method and a literature analysis [12]. It was expected that the characteristic Raman scattering spectrum for weddellite would be obtained with 532 nm excitation. At this photon energy, the autoluminescence effect should be minimal.

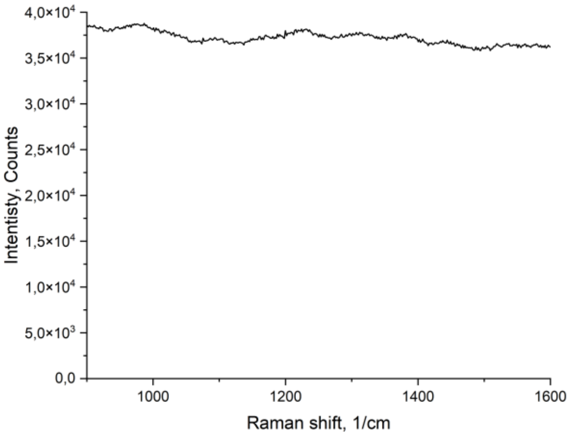

Fig. 7. Raman spectra, excitation 633 nm, sample no. 1, raw data.

Figure 5 shows the recorded Raman spectrum (raw data) with an integration time of 20 seconds and no signal averaging (spectrum accumulation). Figure 6 shows the spectrum after analysis and mathematical-physical processing. This analysis was performed to identify the characteristic peaks of the Raman shift and determine the precise wave vector values. First, the Lorentz peak spectra were matched to the peaks. Then, the spectral baseline was determined and subtracted to highlight the characteristics of the Raman shift spectra. Within the studied spectral range, the following characteristic Raman peaks were observed: 914 cm^{-1}, 1349 cm^{-1}, and 1477 cm^{-1}. The uncertainty for each peak is 1 cm^{-1}. Based on the literature, the substance was confirmed to be calcium oxalate dihydrate [12]. Figure 6 also identifies which vibrational modes of the bonds correspond to the characteristic peaks in the Raman spectrum of weddellite.

The literature [13] also indicates strong autoluminescence of weddellite with 633 nm excitation. In this study, we investigated whether this phenomenon also applies to the present test sample. Figure 7 shows the recorded Raman spectrum (raw data) with an integration time of 20 seconds. As can be seen, this spectrum is characterized by the absence of characteristic Raman peaks. This sample's autoluminescence does not reveal these peaks. Nevertheless, the observation of strong autoluminescence confirms that we are dealing with weddellite.

In conclusion, both SEM and Raman spectroscopy were used to determine the main component of human bone. However, it should be noted that these methods are only applicable to surface studies. A kidney stone is not homogeneous throughout its volume. To fully identify its type, studies of its individual layers are necessary, such as filing it down and carefully inspecting the exposed surface. Further stages of the work are planned to use the RS technique with 785 nm excitation to eliminate the autofluorescence phenomenon and obtain clearer spectra. Additionally, SERS substrates, which exhibit Raman signal enhancement properties, were planned to be used. The research methodology described in this paper requires further development. In addition to the Raman spectroscopy (RS) and scanning electron microscopy (SEM) methods used, the study should be complemented by the Fourier-transform infrared spectroscopy (FTIR) technique.

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