

IDENTIFICATION OF THE URINARY STONE COMPOSITION UPON EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY

I. OSWALD, SIMONA CAVALU, T.T. MAGHIAR, DIANA OSVAT

Faculty of Medicine and Pharmaceutics, University of Oradea, 10, 1 Decembrie Square, 410081
Oradea, e-mail: ioanoswald5@yahoo.com

Abstract. The study was carried out to investigate the composition and type of different urinary stones upon application of extracorporeal shock wave lithotripsy. ATR FTIR spectra revealed the marker bands of a mixed stone composition containing calcium oxalate monohydrate/ calcium carbonate, respectively calcium oxalate monohydrate/cystine. The surface morphology of the samples and elemental analysis was performed by SEM-EDAX confirming the presence of oxalate, carbonate and cystine in the samples. Combination of FTIR spectroscopy and SEM-EDAX allowed quantitative and qualitative evaluation of components, the spatial distribution and the percent of major and trace elements present in a single sample.

Key words: urinary stones, lithotripsy, FTIR spectroscopy, SEM-EDAX.

INTRODUCTION

With the development of advanced instruments and techniques, minimally invasive surgical procedure has gradually replaced open surgery for treating proximal ureteral stones. Since the first successful application of extracorporeal shock waves for lithotripsy (ESWL) of renal stones in 1980, the use of shock wave therapy has rapidly expanded in medicine. For the first time in 1985, extracorporeal shock waves were used for the fragmentation of gallstones. Today, besides treating renal stones and gall stones, also salivary stones, pancreatic stones, nonunion of long bones, epicondylitis humeri radialis, plantar fasciitis, and calcified tendinites of the shoulder are being treated with varying success rates [11]. Urolithiasis is a common, recurring disorder and certain intrinsic and extrinsic factors may be linked in the genesis of urinary calculi. It is a heterogeneous agglutination of various ingredients and a knowledge of the chemical composition of urinary stones helps in understanding the pathogenesis, thereby providing guidelines for proper medical management including adaptation of suitable prophylactic measures.

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The highly variable composition of urinary calculi has led to the development of many different methods of calculi analysis. Reliable analytical information is fundamental for a study of the etiology of formation of stones and is required for planning the policy of medical management. In general, analytical methods can be divided into chemical and physical methods. These include biochemical analysis, X-ray diffraction, polarization microscopy, infrared/Raman spectroscopy, scanning electron microscopy with energy dispersive X-ray analysis, transmission electron microscopy, computed tomography, magnetic resonance imaging and thermo gravimetric analysis [10]. In the present study, FTIR analysis was applied for two different types of stones after ESWL procedure, followed by Scanning electron microscopy with elemental distribution analysis (SEM-EDAX) which is a very important tool in assessing renal stone composition. Combination of FTIR spectroscopy and SEM-EDAX allowed quantitative and qualitative evaluation of components, the spatial distribution and the percent of major and trace elements present in a single sample [4, 7].

MATERIALS AND METHODS

Two different urinary stones naturally removed upon ESWL procedure were examined by FTIR spectroscopy and SEM-EDAX. The shock wave parameters for lithotripsy were as follows: generator type electrohydraulic, maximal focus pressure (p_+) is 41 MPa to 21 kV, energy shock wave per pulse (8 mJ), coupling of the shock wave source to the body is dry (water cushion).

The stones were washed with distilled water, air dried and FTIR spectra were recorded using a Perkin Elmer Spectrum BX FTIR spectrometer equipped with an Attenuated Total Reflectance (ATR) Miracle accessory. The development of diamond as an ATR material has opened up a number of sampling opportunities in the mid infrared spectroscopy. The high refractive index ensures that for incidence angles of 45 degrees, a typical infrared penetration of around 2 μm is achieved. The extraordinary stable covalent bonding structure of diamond accounts for its hardness and physical strength. Consequently, single reflection measurements are capable of yielding good quality spectra under conditions of good optical contact of the sample with the ATR crystal. Hence, the principal benefit is that ATR sampling for abrasive or chemically hostile samples is now routine. The FTIR spectra of both samples were recorded in the region 3000–400 cm^{-1} , operating in reflectance mode with 4 cm^{-1} resolution, and scanning speed of 32 cm/min . A total of 128 scans were accumulated for each spectrum. The surface morphology of the stones was analyzed using a scanning electronic microscope type 5600 LV Jeol equipped with an X-rays spectrometer type Oxford Instrument, with the following characteristics: resolution 3.5 nm with secondary electrons; enlargement 300,000x; local

quantitative chemical analyses based upon the X-rays characteristic spectrum (EDAX) for the elements listed between boron and uranium, with the detection limit of 0.01%; low vacuum conditions 23 Pa.

RESULTS AND DISCUSSION

The identification of the renal calculi composition is essential as it provides information that could be useful for practitioners to find out the underlying cause of kidney stone formation and to decide whether to treat the patients therapeutically or surgically. A study of the chemical composition of renal stones is important for understanding their etiology as well, permitting a proper management of the disease and the prevention of its recurrence. From the recorded FTIR spectra of both samples (Fig. 1 and Fig. 2), the chemical constituents of renal stones were identified and compared with the previously reported values [2, 9]. Based on the spectral characteristics (Fig. 1), the first sample was identified as calcium oxalate monohydrate (or whewellite). The marker bands of this spectrum are located around 1600 cm^{-1} and 1307 cm^{-1} corresponding to asymmetric and symmetrical stretching of the O–C=O bond [6, 9]. The confirmation of calcium oxalate is made by the presence of the two discrete peaks at 948 cm^{-1} and 888 cm^{-1} corresponding to the O–C=O bending mode of vibration. According to the literature [5], the intense band at 774 cm^{-1} assigned to C=O asymmetrical stretching is important for distinguishing calcium oxalate monohydrate from calcium oxalate dehydrate. A small amount of calcium carbonate is also detected in this sample through the presence of the bands at 1462 cm^{-1} (C–O stretching) assigned in literature to calcite form, respectively 1090 cm^{-1} and 644 cm^{-1} assigned to aragonite form. The general features of the spectrum presented in Fig. 1 indicate that the first sample is a mixture of calcium oxalate monohydrate (predominantly) and calcium carbonate. Calcium oxalate stones mainly develop due to hyperoxaluria, which is a metabolic disorder that causes the stone formation [2]. Oxalate is an end product of several metabolic pathways (including those involved in serine, glycine, hydroproline and ascorbate). 10 to 20% urinary oxalate is derived from dietary sources and food rich in oxalate are cranberries, spinach, chocolate and tea [1, 2]. In Fig. 2 are presented the characteristic features of a mixed stone containing calcium oxalate monohydrate and cystine. The fingerprints of oxalate are located in this spectrum at 987 , 870 and 779 cm^{-1} , but the intensity of these bands is drastically reduced compared with those of the previous sample. This spectrum exhibited also the specific bands of a protein, identified as cystine. The strong band located around 1650 cm^{-1} is assigned to C=O asymmetrical stretching vibration weakly coupled with C–N stretching and in plane N–H bending (amide I), 1546 cm^{-1} and 1297 cm^{-1} assigned to amide II and amide III respectively [3, 8]. The higher wavenumber region of the spectrum is dominated by the strong band at 2357 cm^{-1} corresponding to N–H and C–H stretching. Cystine stones are produced by an inherited disorder of the transport of amino acid cystine that results in excess of cystine in the urine

(cystinuria). Cystine crystals are unusually identified in the urinary deposits. The main problem is recognizing cystine by FTIR as a component in mixture of stones due to the similarity of wavelengths of cystine with that of whewellite and uric acid [7]. Even though it is difficult to find out the presence of cystine molecule in FTIR, it is possible to recognize it through EDAX and it will be possible to confirm the presence of cystine in mixed urinary stones.

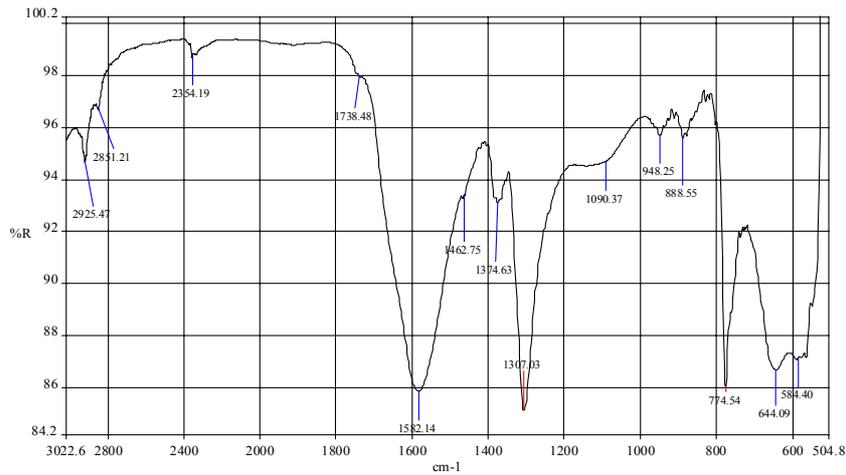


Fig. 1. ATR FTIR spectrum of mixed kidney stone containing calcium oxalate monohydrate and calcium carbonate.

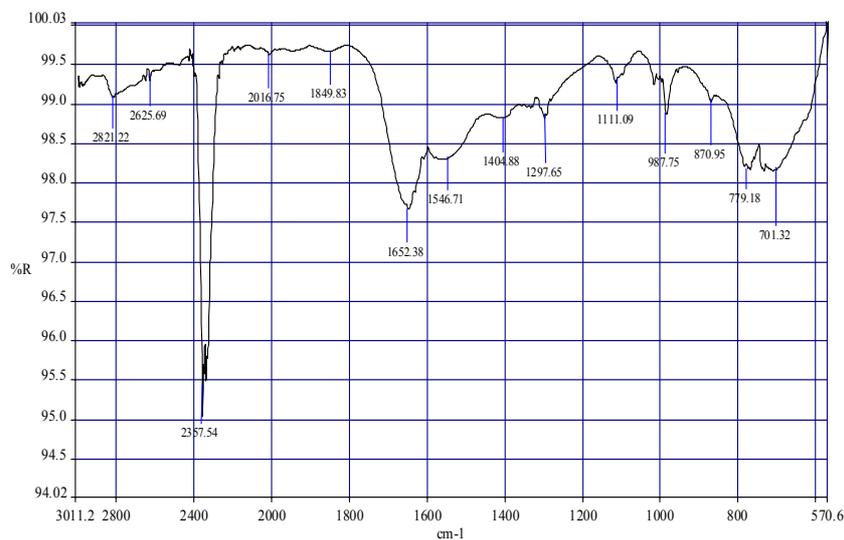


Fig. 2. ATR FTIR spectrum of mixed kidney stone containing cystine and calcium oxalate monohydrate.

In order to elucidate this aspect of mixed composition of some urinary stones, the findings of FTIR were correlated with SEM-EDAX and detailed data generated. Using SEM-EDAX, the spatial distribution of major and trace elements were studied to understand their initiation and formation.

Fig. 3 presents the surface morphology of the mixed kidney stone containing calcium oxalate monohydrate / calcium carbonate (a) and cystine/calcium oxalate monohydrate (b) respectively, along with the quantitative results of the elemental analysis. In the first case, the surface morphology indicates two different crystallization regions, the dark colored region showing a reduced calcium content as was revealed by the spatial distribution (not shown in this figure). The elemental analysis confirmed the presence of oxalate structures, indicated by the percentage of different elements present in a single sample. On the other hand, the uncertainty regarding the presence of cystine or uric acid in the second sample (Fig. 3b) is eliminated by the evidence of sulphur in elemental analysis, as cystine structure is characterized by $-S-S-$ bonds. The surface morphology in this case also confirms the existence of two different crystallization regions, the spatial distribution indicating that sulphur is concentrated in the massive, parallelepiped grey-colored structure, while the white colored deposits on the surface consist mainly of calcium and trace elements. The data obtained by SEM-EDAX analysis are in concordance with FTIR spectral features and with previously reported results in literature.

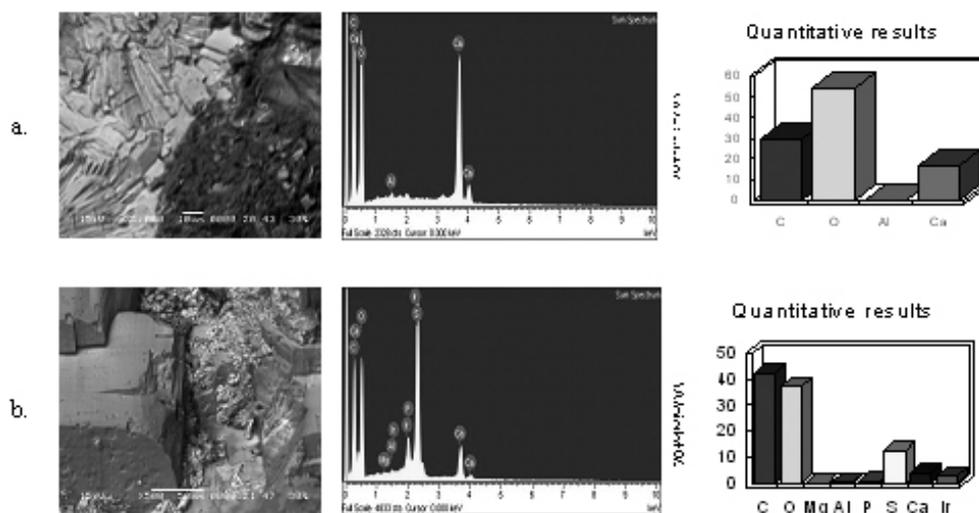


Fig. 3. The morphology of the mixed kidney stone containing: a. calcium oxalate monohydrate / calcium carbonate, and b. cystine / calcium oxalate monohydrate respectively, and the corresponding EDS spectra of the compound elements.

CONCLUSIONS

The accurate analysis of renal stones by combining FTIR spectroscopy with SEM-EDAX analysis could definitely be helpful in understanding the genesis of calculi formation. Our study was carried out in order to investigate the composition of two different urinary stones upon application of extracorporeal shock waves for lithotripsy. Identification of calcium oxalate monohydrate/calcium carbonate and respectively cystine/calcium oxalate monohydrate mixed stones was made upon comparing the fingerprint region of the FTIR spectra with the existing data in literature. The confirmation of the FTIR results was sustained by the quantitative evaluation of components through SEM-EDAX analysis. Even though it is difficult to find out the presence of cystine molecule in FTIR, it is possible to recognize it through EDAX and to confirm the presence of cystine in mixed urinary stones.

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