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MUHAMMAD HUSSAN KHASKHELI

SYED TUFAIL HUSSAIN SHERAZI

HUMA MAZHAR UJAN

SARFARAZ AHMED MAHESAR

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# Transmission FT-IR spectroscopic analysis of human kidney stones in the Hyderabad region of Pakistan

Muhammad Hassan KHASKHELI<sup>1</sup>, Syed Tufail Hussain SHERAZI<sup>2,\*</sup>, Huma Mazhar UJAN<sup>1</sup>, Sarfaraz Ahmed MAHESAR<sup>2</sup>

<sup>1</sup>*Department of Biochemistry, Shah Abdul Latif University, Khairpur-PAKISTAN*

<sup>2</sup>*National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro 76080-PAKISTAN*

*e-mail: tufail.sherazi@yahoo.com*

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The purpose of the present study was to investigate the composition of kidney stones removed from human patients. Seventeen samples were collected after surgery (5 females and 12 males; age range 2 to 60 years). The samples were analyzed for composition by transmission Fourier transform infrared (FT-IR) spectroscopy. The 17 analyzed samples of kidney stones comprised 29.4% calcium oxalate, 29.4% uric acid, 29.4% calcium oxalate (CaOx)-uric acid, 5.9% phosphate, and 5.9% calcium oxalate-phosphate stones. The results of the study indicated that transmission FT-IR spectroscopy could be used as an environmentally friendly technique for rapid screening of kidney stones.

**Key Words:** Kidney stone, transmission FT-IR, stone composition

## Introduction

Commonly kidney stones are considered to be composed of crystalline constituents and organic matrix.<sup>1</sup> Commonly, urinary stones are present in the form of renal stones, but bladder stones are occasionally found in children living in undeveloped countries. The size and frequency rates of kidney stones may be influenced by nutritional, genetic, and environmental aspects.<sup>2</sup> In the USA and Europe approximately 0.1%-0.4% people suffer from kidney stones every year, while 2%-5%, 8%-15%, and about 20% of the population in Asia, North America, and Saudi Arabia, respectively, are thought to have kidney stones in their lifetime.<sup>3-7</sup> During last 2 decades the rate of renal stones recurrence was about 75%.<sup>8</sup> In some patients, the stones develop due to

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\*Corresponding author

well defined systemic diseases, whereas the pathogenesis of the stones is still unclear in the other cases. The complete history of each kidney stone patient could provide significant information about the stone development and on the basis of data a pharmaceutical approach could be applied to prevent recurrent stone formation.<sup>9,10</sup> Therefore, analysis of the composition of kidney stones is an important step to understand the mechanism of stone formation.

The constituents of kidney stones may be classified by different analytical techniques, e.g. wet chemical methods or by instrumental techniques such as crystallographic methods,<sup>11,12</sup> thermal analysis,<sup>13</sup> and Fourier transform IR (FT-IR) or Raman spectroscopy.<sup>14,15</sup> Chemical methods are comparatively erroneous due to occasional false-positive and false-negative results.<sup>16</sup> Although X-ray diffraction is widely used for quantification of the constituents of kidney stones, it is unable to characterize amorphous species such as carabapatite or struvite.<sup>14–16</sup>

Infrared spectroscopy (FT-IR) is another suitable analytical technique from which information about the composition of kidney stones could be achieved rapidly. Furthermore, the quantification of the relative amount of each constituent present is possible without using any solvent. Therefore, FT-IR spectroscopy has been supposed to be the most appropriate technique for kidney stone analysis. In our previous studies we applied FT-IR spectroscopy for the rapid determination of the different constituents present in the edible oils and pharmaceutical formulations.<sup>17–23</sup> In the present study FT-IR was used for the rapid assessment of kidney stones without using any toxic solvents for digestion or extraction.

## Experimental

### Samples and reagents

The kidney stones for the present study were surgically removed from 17 patients admitted for treatment in Liaquat University Hospital, Jamshoro, Pakistan, during 2007. All kidney stones after surgery were placed on sterile gauze to dry in air, and washed with distilled deionized water to remove the bile and debris. After drying, the kidney stones were cut into small pieces to get representative samples. One quarter portion of the total kidney stone size was ground with a pestle and mortar until a fine homogeneous powder was obtained, which was then stored in a glass tube, and kept in a dark cabinet until analysis.

### Standards

Pure standards of calcium oxalate mono and dihydrate, calcium phosphate, ammonium hydrogen urate, uric acid anhydrous, and ammonium sodium urate were obtained from Sigma-Aldrich (Chemie GmbH). Initially, the FT-IR spectra of all individual standards were acquired. NICODOM IR Kidney Stone Library (NICODOM Ltd., Prague, Czech Republic) containing 1668 transmission FT-IR spectra was installed within the software of FT-IR. Then spectra of each individual and combined standard (mixed with different ratios of most commonly found kidney stones such as oxalates, urates, uric acid and phosphate) were matched with the spectra stored in the library. However, our own spectral library was also developed to differentiate the kidney stone samples with their matching percentages.

## FT-IR spectral measurements

All infrared spectra were obtained using a ThermoNicolet Avatar 330 FT-IR spectrometer equipped with a deuterated triglycine sulfate (DTGS) detector and KBr optics, controlled by OMNIC software (Thermo Nicolet Analytical Instruments, Madison, WI, USA). The pellets of standards and samples were prepared in KBr and spectra were recorded in transmission mode by co-addition of 32 scans at a resolution of  $8\text{ cm}^{-1}$ . To obtain accurate results a fresh background spectrum was subtracted from the FT-IR spectrum of each standard or sample.

A small amount of ground sample (1 mg) of kidney stone was mixed with dried KBr and the mixture was thoroughly mixed by mortar and pestle. In the pellet machine, a pressure of  $12\text{ t/cm}^2$  was applied to form a pellet of approximately 12 mm in diameter. The pellets were placed in the transmission holder in front of the IR beam. The spectra were acquired in the middle IR range, i.e.  $400$  to  $4000\text{ cm}^{-1}$ .

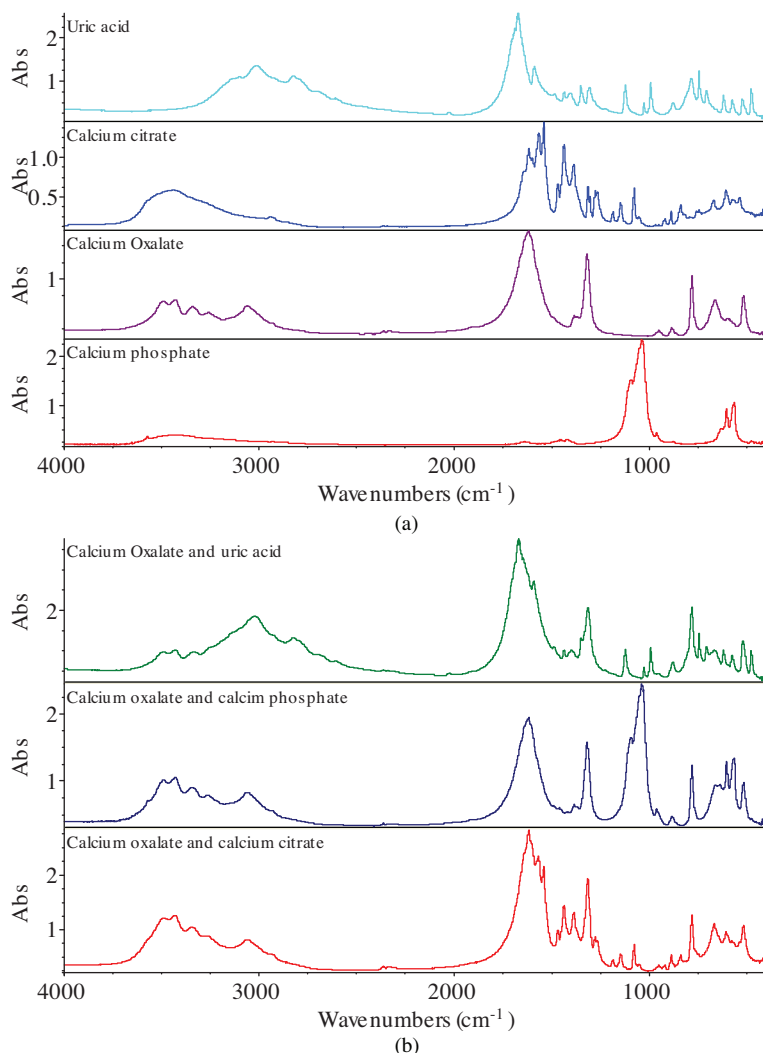
## Results and discussion

Generally, FT-IR spectra show absorption bands (stretching or bending) at special wavelengths, representing particular functional groups as a result of rotational and vibrational motions in molecules. The absorption or intensity of a band is greatly influenced by the molecular composition and structure of each constituent of a complex mixture. Therefore, in the kidney stone each constituent could be differentiated by its absorption spectrum with specific stretching or bending bands. Commonly in FT-IR spectroscopy 2 techniques are applied for the quantitative or improved semiquantitative analysis of mixtures. The partial least square (PLS) technique provides exactitude results for the composition of the analyzed unknown sample. When the range of concentration is very broad and an erratic number of constituents are present PLS is less applicable. The second possible method is the spectral matching technique, through which an unknown sample spectrum is compared to a number of library spectra, installed within the FT-IR and identified by the most similar spectrum. Similarities close to 100 means that the sample consists of the same constituents with the same ratio.

In the present study, all FT-IR spectra of kidney stones were identified with matching percentages of IR spectra of the reference compounds stored in the library. Oxalates were differentiated by a strong band (C=O) at  $1620\text{ cm}^{-1}$  asymmetric to the left, a modest band (C-O) near  $1316\text{ cm}^{-1}$ , and a valence vibration of  $\text{H}_2\text{O}$  in staircase form at  $3500$  to  $2700\text{ cm}^{-1}$  with 2 maxima at  $3490$  and  $3429\text{ cm}^{-1}$ .<sup>18</sup> The distinctive out-of-the-plane deformation peaks were found in the fingerprint region, at  $780\text{ cm}^{-1}$ , and 3 secondary vibrations at  $516$ ,  $883$ , and  $951\text{ cm}^{-1}$  (Figure 1). Oxalate and urate standards exhibited characteristic bands at  $1322$  and  $946\text{ cm}^{-1}$ , respectively.<sup>18</sup> The interpretation of the most important peaks in the infrared spectra was carried out with the help of the literature<sup>18-21</sup> and is presented in Table 1.

Phosphates containing  $\text{PO}_4^{3-}$  ion revealed that the stretching and bending vibrations arise in the mid IR region, i.e.  $900$ - $1200$  and  $300$ - $600\text{ cm}^{-1}$ , respectively. A clear broad IR band occurs at  $985$ - $1100\text{ cm}^{-1}$  with intense feature at  $1038\text{ cm}^{-1}$  due to  $\text{PO}_4^{3-}$  stretching vibration, while a sharp and intense band present at  $605\text{ cm}^{-1}$  is due to the bending vibration of  $\text{PO}_4^{3-}$ . The breakdown of urea in human urine leads to the formation of high carbon dioxide content; eventually this will be converted into amorphous carbon or carbonate species, which may attach to urinary stones.<sup>24</sup> In calcium phosphate type crystals, carbonate ( $\text{CO}_3$ ) ion is

preferred to substitute in  $\text{PO}_4^{-3}$  site rather than in OH site, which gives an IR absorption band at  $872\text{ cm}^{-1}$ . The moderately strong bands at  $1419$  and  $1469\text{ cm}^{-1}$  and weak bands at  $1416$  and at  $1468\text{ cm}^{-1}$  are because of  $\text{CO}_3$  ion. Additionally, a weak band observed at  $1550\text{ cm}^{-1}$  confirmed the presence of carbonate groups substituted in OH sites.<sup>24</sup> A very strong band extending from  $3250$  to  $3520\text{ cm}^{-1}$  with a peak at  $3429$  and a shoulder at  $2800\text{ cm}^{-1}$ , which arises due to the stretching of the O–H functional group of uric acid, was also observed as reported in earlier studies.<sup>24–27</sup>



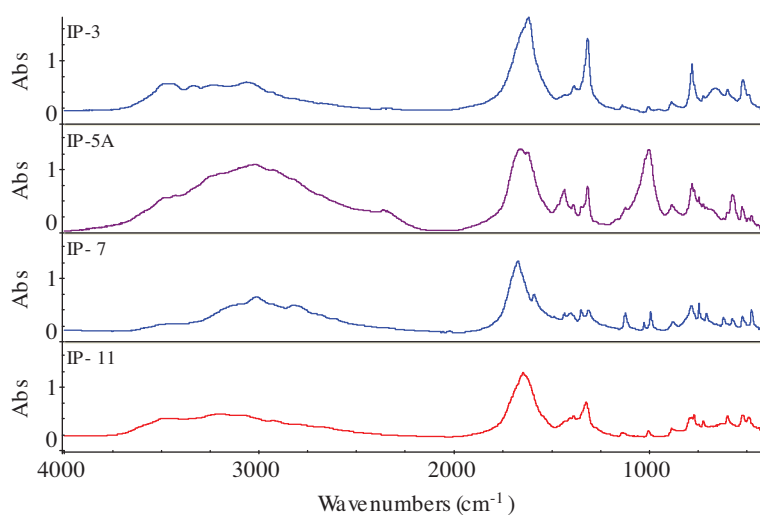
**Figure 1.** Stacked FT-IR spectra of the pure and mixed kidney stone standards (1A and 1B).

Figure 1A and 1B show the stacked FT-IR spectra of the pure and mixed kidney stone standards.

Figure 2 shows representative stacked FT-IR spectra of a few kidney stone samples. Above 95% matching of sample spectra with standards stored in the library was accepted as showing their presence in the kidney stone samples. An independent test set containing pure and mixed standards was used for validation of the quality of the spectral matching model. Satisfactory results were achieved, which is very obvious from the matching of standards spectra (Figure 1) with samples of kidney stones (Figure 2).

**Table 1.** IR spectral data ( $\text{cm}^{-1}$ ) of kidney stone standards.

Peaks	Assignments [18-21]
604-605 s	$\text{PO}_4^{3-}$ bending
780-781 vw	C-C stretch
871-872 w	$\nu_3\text{CO}_3$
1038-1031 vvs br	$\nu_3\text{PO}_4^{3-}$ stretch $\nu_1\text{CO}_3^{2-}$
1315-1316 w	C-C vibr
1416-1419 m	$\nu_3\text{CO}_3$
1468-1469 m	$\nu_3\text{CO}_3$
1550-1551 m	C=C, $\nu_3\text{CO}_3$
1663-1665 m	OH deformation $\nu(\text{C}=\text{O})$
1995-1997 vw	CO vibrations
2800-2805 sh	$\nu(\text{CH}_3)$ asym
3422-3429 sbr	$\nu$ OH stretch

**Figure 2.** Representative stacked FT-IR spectra of kidney stone samples.

According to the results shown in Table 2, the occurrence of kidney stones was dominant in the male patients (70% vs. 30%). The analyses of 17 kidney stones was as follows: the leading type was calcium oxalate stones (29.4%), then uric acid stones (17.6%), ammonium urates (11.8%), followed by mixed phosphate and uric acid stones (23.6%), and mixed oxalate with carbonate stones (17.6%).

Comparatively, in male patients, the frequency of calcium stones was greater, while uric acid stones predominated in female patients. Among the calcium-containing stones, calcium oxalate stones (50%) were the most common, followed by calcium oxalate mixed with uric acid (30%), calcium oxalate mixed with phosphate (10%), and carbonate (10%).

Similarly, among the calcium-containing kidney stones, calcium oxalate monohydrate stones (40%) were

predominant. These results indicated that hyperoxaluria is particularly common in stone patients and perhaps it is one of the main sources of calcium oxalate stone creation.

**Table 2.** Summary of kidney stones with their composition assessed by FT-IR.

Patient code	Age (years)	Sex	Composition of stone
IP-01	02	Male	Ammonium urate
IP-02	02	Male	Uric acid + calcium oxalate monohydrate
IP-03	18	Male	Calcium oxalate monohydrate
IP-04	50	Female	Uric acid
IP-05	60	Male	Magnesium ammonium phosphate
IP-06	07	Female	Uric acid
IP-07	04	Female	Uric acid
IP-08	02	Male	Calcium oxalate monohydrate + uric acid
IP-09	12	Male	Calcium oxalate monohydrate
IP-10	12	Male	Uric acid + calcium oxalate monohydrate
IP-11	08	Female	Uric acid + calcium oxalate monohydrate
IP-12	43	Male	Calcium oxalate monohydrate
IP-13	45	Male	Calcium oxalate monohydrate
IP-14	03	Male	Ammonium urate
IP-15	33	Male	Calcium oxalate monohydrate + calcium carbonate
IP-16	20	Female	Magnesium ammonium phosphate+ calcium oxalate dihydrate
IP-17	48	Male	Calcium oxalate dihydrate

## Conclusion

In the present study, FT-IR spectroscopy was applied for the assessment of the composition of 17 kidney stones just by matching the spectra of kidney stone samples with the stored spectra in the library. Some kidney stone standards were also run to verify the results of matching and were found to be satisfactory. The time required for the analysis of a single stone by FT-IR was less than 1 min. Furthermore, a small amount of sample (1-2 mg) was used to record the FT-IR spectra and several components of kidney stones were simultaneously detected through a single run.

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