



FTIR spectroscopic analysis of renal stones in the population of District Khairpur, Sindh, Pakistan

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Abstract

The aim of current study was to examine the composition of kidney stones laproscopically detached from the patients in District Khairpur, Sindh, Pakistan. The 32 kidney stone samples were collected after surgery of patients (22 males and 10 females in between the age of 15 to 80 years) admitted for the treatment in Khairpur Medical College, Civil Hospital Khairpur during 2018 to 2019. The calculi specimens were analyzed for composition by FTIR spectroscopy. The 32 investigated specimen of renal calculi contained 31.25% of calcium oxalate monohydrate (CaOx), 6.25% calcium oxalate dihydrate, 12.5% of Uric acid (pure), 6.25% of ammonium urate, 6.25% of magnesium ammonium phosphate (struvite), and in mixed stones 28.13% of calcium oxalate monohydrate + uric acid stones and 9.37% of magnesium ammonium phosphate + calcium oxalate stones. Calcium oxalate monohydrate either singly (31.25%) or in combination with Uric acid (28.13%) was the leading constituent of Kidney stones. Investigation of kidney stones based on FTIR suggests that Calcium oxalate monohydrate individually or in combined state with uric acid was the most predominant component of human renal calculi in the patients of district Khairpur, Sindh, Pakistan.

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Introduction

Kidney stones are solid materials created by the urinary system (Bahmani *et al.*, 2016). Kidney stone is a worldwide circulated old disease (Sakhaee, 2018) that has very much puzzled human beings as well as doctors for centuries. Kidney stone is a foremost human health issue and is going to take a significant place in every day medical practice. The epidemiological study shows that kidney stone is a multifactor disease (Channa *et al.*, 2007; Shabsoug, 2003). Age, sex, diet, weather, race and heredity are serious risk aspects (Wróbel & Kuder, 2019). Kidney stone is actually a disease of all ages (Shabsoug *et al.*, 2016). Kidney stone may root two difficulties 1, when it changes its place 2, when it grows to interrupt kidney function and harm occurs (Rafique *et al.*, 2000). The clinical approach to the calculi formers involve medical as well as surgical problems (Shafi *et al.*, 2016). In Pakistan, renal stone is the 6th utmost common surgery demanding situation. The purpose of treatment comprises elimination of stone, removal of infection, conservation of kidney function and stoppage of recurrence. Dealing with kidney stone disease, urologists are still very much puzzled to select the best therapy in this new era. In previous days the open stone surgery percentage was stated as 26% in Pakistan, at present it is 8% and 3-5% in U.S. This surgical procedure seems to be the most favored remedy in undeveloped and rural areas of Sindh.

The investigation of the chemical composition of kidney stones removed by open surgery play a key role in the etiopathogenesis, analysis and cure of variety of renal calculi (Alelign & Petros, 2018). Unfortunately, this vital practice is ignored by doctors and surgeons in our hospitals. Different methods are being used for the investigative determination of renal stone comprising x-ray diffraction (Hidas *et al.*, 2010) and wet chemical investigation test (Hashim & Zawawi, 1999). By results and accuracy IR has preference over other techniques, like X-ray diffraction and electron microscopy. A wet chemical investigation is often used in hospital laboratories to analyze different components in renal stones and reasons of their formation.

This technique has few defects also that the rare material is missed and cannot be utilized for other analytical purposes (Hashim & Zawawi, 1999; Khaskheli *et al.*, 2012).

The best method for the analysis of calculi must be selective, accurate, rapid and of low-cost. It must be proficient to initiate the facts of its composition and structure. The importance of analysis of kidney stone by FT-IR is increasing because it is rapid, takes < 1 minute, is required a little amount of calculi in the powder form. By results and accuracy IR has preference over other techniques (Dao NQ, Daudon M. 1997; Khaskheli *et al.*, 2012).

Renal stone is a reiterating disease (Zisman, 2017). In history, kidney stone victims who had a single stone will grow other one (Riordan *et al.*, 2009; Samad *et al.*, 2017). Recent clinical methods have made this retelling kidney stone disease “an escapable illness in 95% of victims. Kidney stone is a growing problem in Sindh and no study for its compositional and structural analysis through FT-IR has emerged from here. Due to the increasing number of kidney stone victims in our hospitals and severe complications of this problem, it is very necessary to work hard over this significant health problem in the district Khairpur, Sindh, Pakistan.

Materials and methods

Patient population

There were 32 kidney stone patients, 22 males and 10 females (in between the age 15 to 80 years) at Khairpur Medical college civil hospital Khairpur, as shown in Table 1 and Table 2. All our patients were of low socioeconomic background and resident of district Khairpur, Sindh, Pakistan. They used Pakistani common food which includes local vegetarian menu with pulses and very low amount of meat mixed with rice and wheat.

Stone Analysis

The materials for this study were kidney stones eliminated through operation from 32 patients during years 2018 to 2019.

All renal calculi removed during operation were put on sterilized gauze to air dry and then washed carefully with double distilled water (to eliminate organic matter) and dried in oven at 37° C properly, then the weight of the samples was obtained. After observing the structural features such as colour, size and shape, single kidney stone from each patient was cut into pieces properly and crushed with a pestle and mortar for 5 minutes. Obtained fine homogeneous powder of stone samples was then stored in a sample tube, kept in dark cabinet until analyzed. The composition of the stone powder was determined by using FTIR spectrophotometer (NICOLET iS10).

FT-IR spectroscopy

FT-IR is very much favored and widely used technique to study urolithiasis. This is an appropriate analytical method for the compositional characterization of renal calculi. It is quick and accurate and offers qualitative and semi-quantitative investigation. Moreover, the quantification of the relative quantity of each component available is possible deprived of utilizing any solvent. The benefits of FT-IR over the dispersive methods are speed, accuracy, internally calibrated, mechanical ease and quality assurance applications.

Standards

Standards like $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$, $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$, $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$, CaCO_3 , uric acid and xanthine of good quality were bought from E. Merck (West Germany). All powder form specimens were analyzed by a well established method. Firstly, the FT-IR spectra of all standards were obtained individually. Then the spectra of all the joint and single standards were compared with the spectra saved in the library.

Results and discussion

In general, FT-IR spectrum show absorption bands at specific wavelength, which represent certain functional groups due to the rotations and vibrations within the molecules. The strength of a band is hardly affected by the structure and composition of each component of a complex. Hence, in the renal calculi each component can be distinguished by the definite bends formed in its absorption spectrum. Generally, in FT-IR

spectrophotometry there is a spectral matching method, by which an unidentified specimen spectrum is matched to the spectra, fixed in the FT-IR and recognized by the most related spectrum. Resemblances near to 100% shows that the specimen contains the identical components with similar proportion.

Table 1. Age and sex wise data of the kidney stone patients.

Sex Wise		
Sex	Frequency	Percentage%
Male	22	68.75
Female	10	31.25
Total	32	100
Age Wise		
Age group	Frequency	Percentage%
15- 30	7	(21.87%)
31 – 45	13	(40.6%)
46 – 60	10	(31.25%)
61 & above	2	(6.25%)
Total	32	(100%)

Table 2. FT-IR investigation of renal calculi with their chemical composition.

Specimen Code	Composition of Stone	Gender	Age (years)
KS-01	Ammonium Urate	Male	18
KS-02	(Ca ox) $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$	Male	20
KS-03	(Ca ox) $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$	Male	50
KS-04	Uric acid	Female	60
KS-05	Calcium oxalate monohydrate + Uric acid	Male	42
KS-06	$\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$	Male	55
KS-07	Ammonium urate	Male	60
KS-08	Magnesium Ammonium Phosphate	Female	43
KS-09	Calcium oxalate dehydrate	Female	45
KS-10	(Ca ox) $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$	Male	33
KS-11	(Ca ox) $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$	Male	25
KS-12	Calcium oxalate monohydrate + Uric acid	Male	28
KS-13	(Ca ox) $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$	Male	46
KS-14	Uric acid + Calcium oxalate monohydrate	Female	52
KS-15	(Ca ox) $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$	Male	58
KS-16	Calcium oxalate monohydrate + Uric acid	Male	34
KS-17	Mg Ammonium Phosphate + Ca oxalate dehydrate	Female	29
KS-18	(Ca ox) $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$	Male	38
KS-19	Uric acid	Female	40
KS-20	Uric acid + Calcium oxalate monohydrate	Male	73
KS-21	Uric acid	Female	56
KS-22	(Ca ox) $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$	Male	36
KS-23	(Ca ox) $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$	Male	44
KS-24	Mg Ammonium Phosphate + Ca oxalate dehydrate	Female	22

KS-25	(Ca ox) $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$	Male	24
KS-26	Calcium oxalate monohydrate + Uric acid	Male	35
KS-27	Mg Ammonium Phosphate + Ca oxalate dehydrate	Male	47
KS-28	Uric acid	Female	36
KS-29	(Ca ox) $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$	Male	45
KS-30	Calcium oxalate monohydrate + Uric acid	Male	43
KS-31	Calcium oxalate monohydrate + Uric acid	Female	60
KS-32	Uric acid + Calcium oxalate monohydrate	Male	71

Table 3. Frequency and (%) of stones with composition.

Chemical composition of kidney stone	Frequency Percentage	
Calcium oxalate monohydrate	10	31.25%
Calcium oxalate dehydrate	2	6.25%
Uric acid (pure)	4	12.5%
Ammonium urate	2	6.25%
Magnesium Ammonium Phosphate (Struvite)	2	6.25%
Uric acid + Calcium oxalate monohydrate	9	28.13%
Calcium oxalate + Struvite	3	9.37%
Total	32	100%

In current study, all FT-IR spectra of renal calculi were recognized with comparing percentages of IR spectra of the reference compounds saved in the library.

Investigation revealed that calcium oxalate monohydrate (whewellite) was found in 10 (31.25%) of urinary stones and showed spectral bands at $1600 - 1610 \pm 10$, $1300 - 1310 \pm 10$, $780 - 785 \pm 10 \text{ cm}^{-1}$. This spectrum was matched with the standard of $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$. The spectrum peaks of both, the specimen and standard were quite similar (Fig. 2. B). Calcium oxalate dihydrate was found in 2 renal stones (6.25%) and showed the spectral bands at $1600 - 1610 \pm 10$, $1300 - 1310 \pm 10$, $780 - 785 \pm 10 \text{ cm}^{-1}$. The specimen spectrum was compared to that of the standard, both of them have similar peaks (Fig. 3. B). The 2(6.25%) urinary calculi were of MgNH_4PO_4 and showed the spectral bands at $1480 - 1490 \pm 10$, $980 - 990 \pm 10$, $750 - 760 \pm 10 \text{ cm}^{-1}$. The specimen spectrum of phosphate was also compared with the standard of MgNH_4PO_4 , both showed same peak identification (Fig. 4. B). The 4(12.5%) calculi were of uric acid and showed spectral bands in cm^{-1} $2990 - 3000 \pm 10$, $2800 - 2810 \pm 10$, $1610 - 615 \pm 10$, $1290 - 1300 \pm 10$, $1115 - 1120 \pm 10$, $985 - 990 \pm 10$ and $750 - 765 \pm 10 \text{ cm}^{-1}$. The uric acid specimen spectrum was matched with the standard, both sample and standard showed the similar peaks (Fig. 5. B).

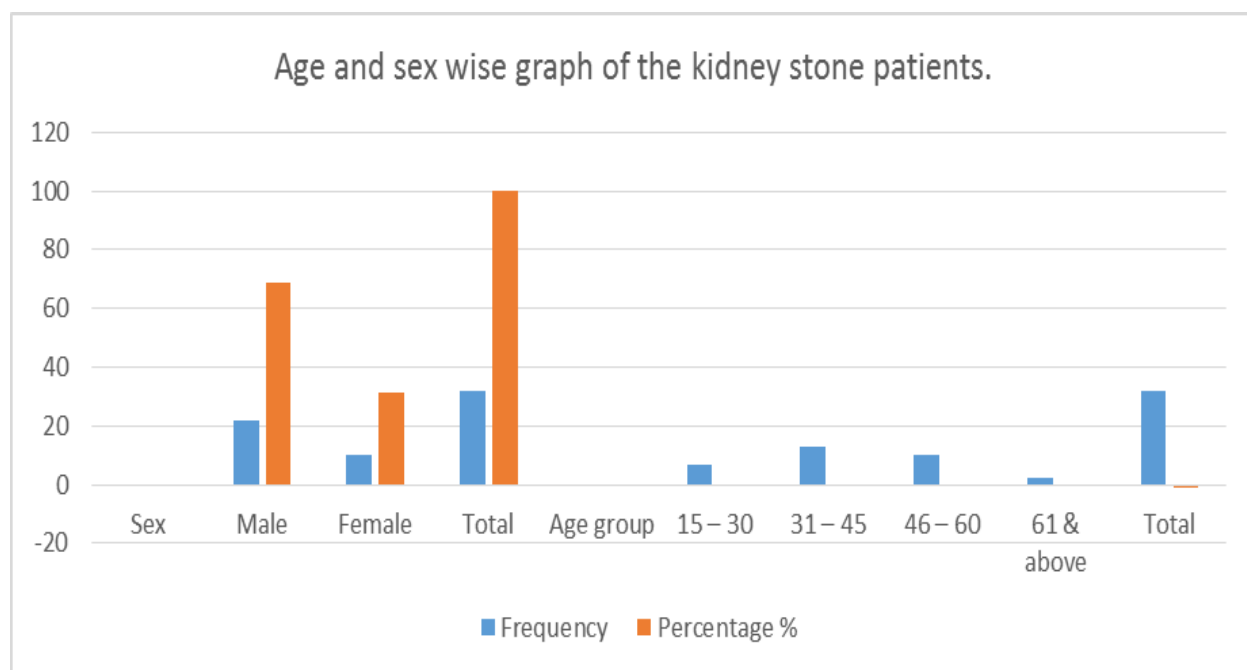


Fig. 1. Age and sex wise graph of kidney stone patients.

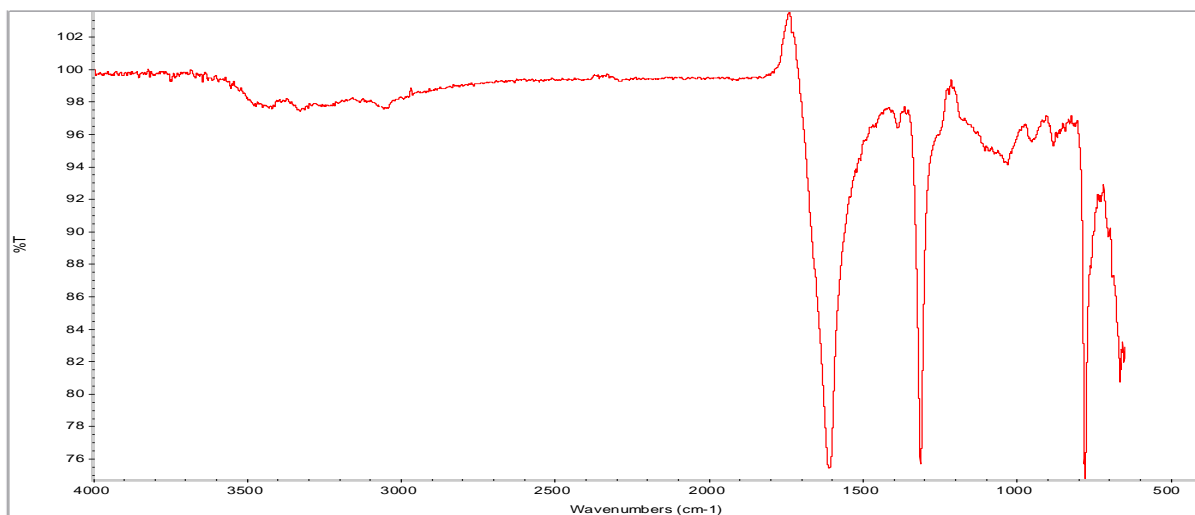


Fig. 2(A). Showing typical FT-IR spectra of (Ca Ox) CaC₂O₄ · H₂O stone standard.

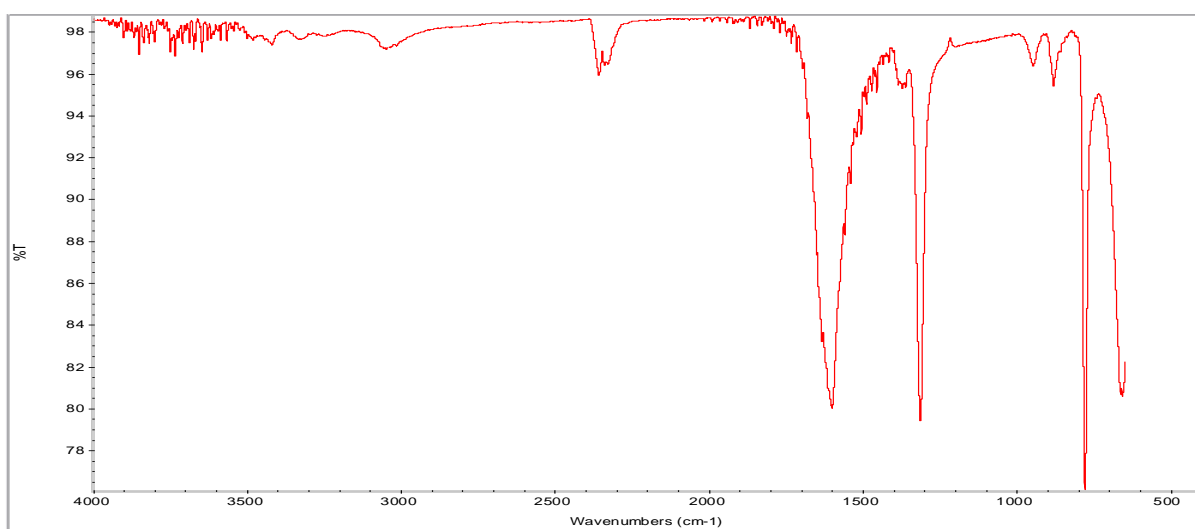


Fig. 2(B). Showing typical FT-IR spectra of (Ca ox) CaC₂O₄ · H₂O stone sample.

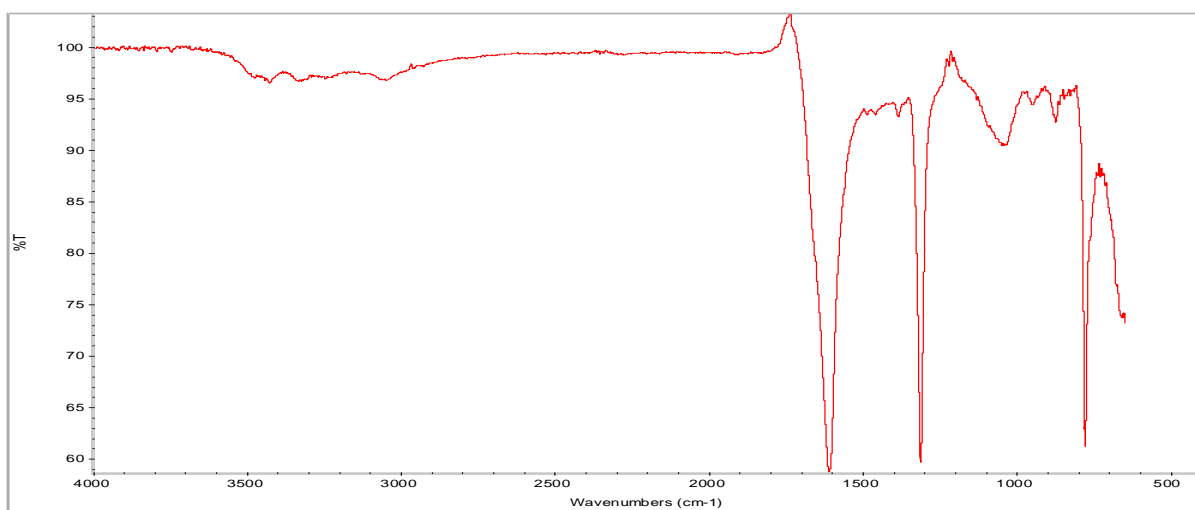


Fig. 3(A). Showing typical FT-IR spectra of (Ca ox) CaC₂O₄ · 2H₂O stone standard.

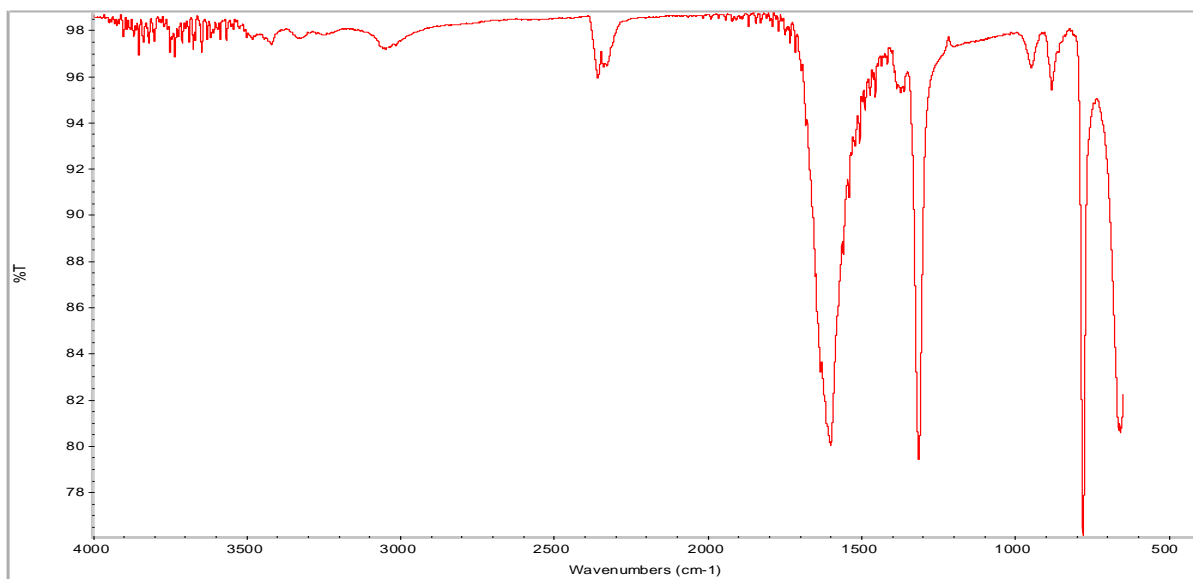


Fig. 3(B). Showing typical FT-IR spectra of (Ca ox) $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ stone sample.

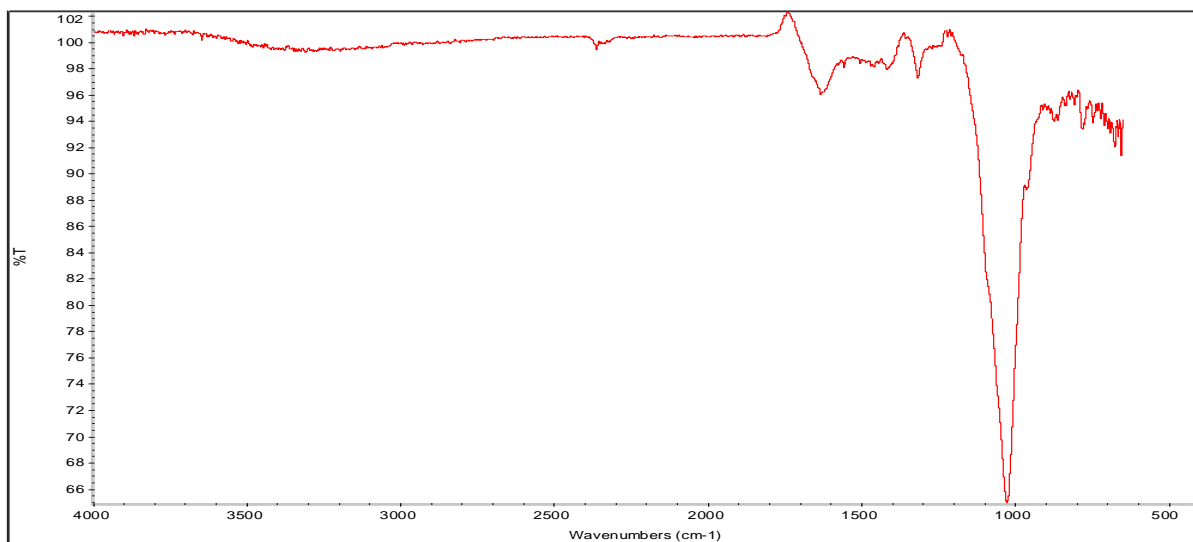


Fig. 4(A). Showing typical FT-IR spectra of $\text{MgNH}_4 \cdot \text{PO}_4 \cdot 6\text{H}_2\text{O}$ stone standard.

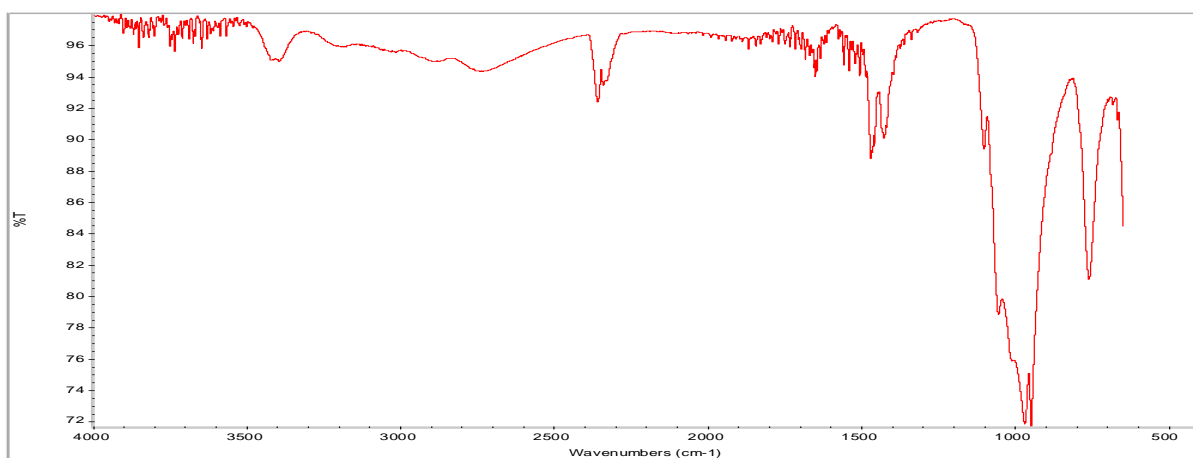


Fig. 4(B). Showing typical FT-IR spectra of $\text{MgNH}_4 \cdot \text{PO}_4 \cdot 6\text{H}_2\text{O}$ stone sample.

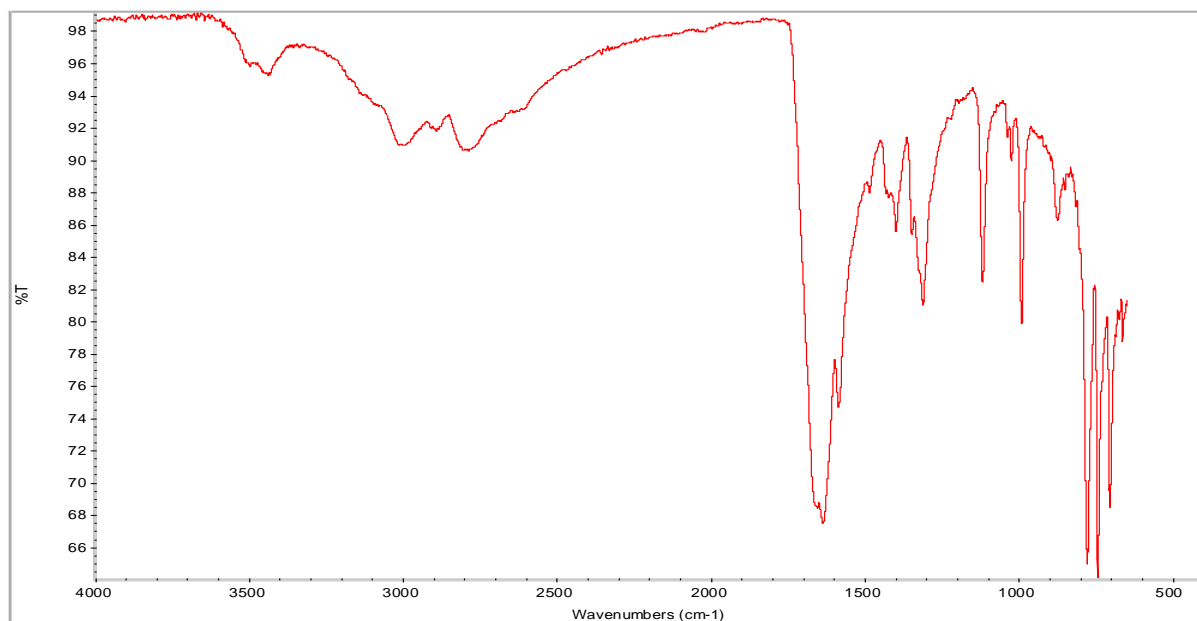


Fig. 5(A). Showing typical FT-IR spectra of uric acid stone standard.

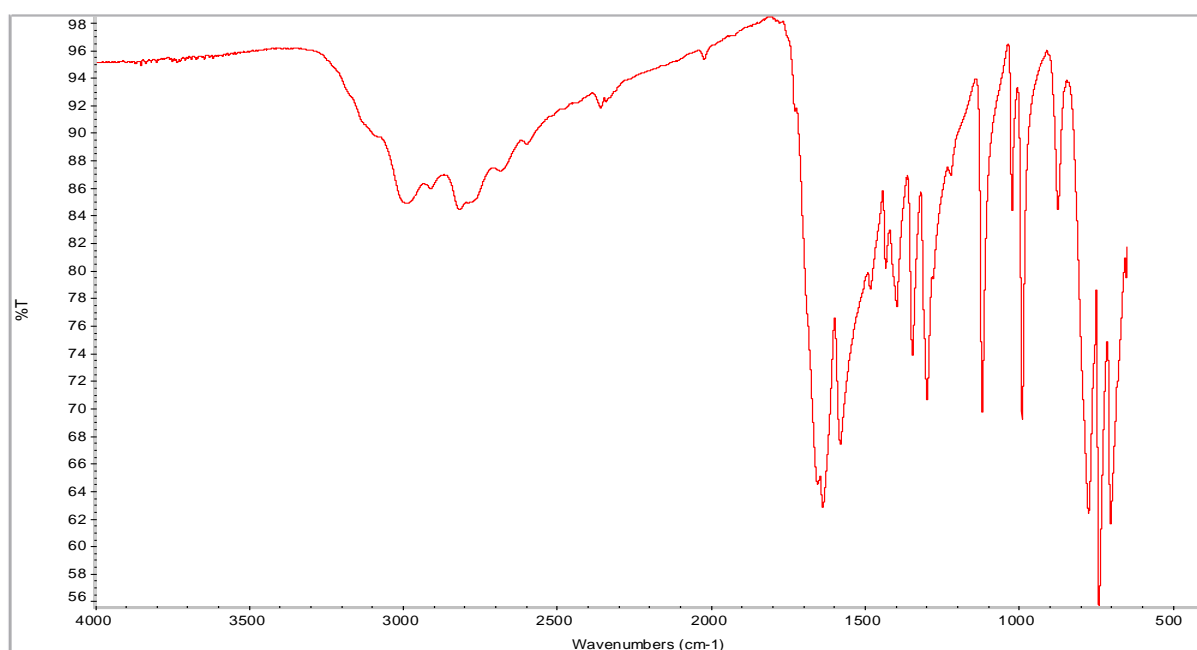


Fig. 5(B). Showing typical FT-IR spectra of uric acid stone sample.

Conclusion

In current study, FT-IR spectrophotometry was used for the evaluation of the composition of 32 renal calculi by comparing the spectra of renal calculi specimen with the saved spectra in the collection of FTIR. The standards of renal calculi were also run to validate the results of comparing and found acceptable. The time needed for the investigation of a calculi specimen by FT-IR was

not more than 1 min. Moreover, a minute quantity of specimen (0.5-2 mg) was utilized to obtain the FT-IR spectra and various constituents of renal calculi were identified at the same time through a single run. The investigation revealed that calcium oxalate mono hydrate either alone or in combined state was the most common constituents of renal calculi of the 32 patients of district Khairpur.

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