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SYMPOSIUM PAPER

Problem in analyzing cystine stones using FTIR spectroscopy

Y. M. Fazil Marickar · P. R. Lekshmi · Luxmi Varma · Peter Koshy

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Abstract 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 calculi in urinary tract present a significant problem in patients. We have recorded that cystine calculi are very uncommon in our region. Cystine crystals are unusually identified in the urinary deposits. The problem of recognizing cystine by FTIR as a component in mixture of stones is significant. The problem is compounded by the similarity of wavelengths of cystine with that of whewellite and uric acid. The objective of this paper is to elucidate the problems of identifying cystine in stone analysis and identifying a solution to get over this deficiency. Out of 1,300 urinary stones analysed by ordinary wet chemical methods and infrared spectroscopy, 30 stone samples, which were reported to have cystine peaks in significant numbers, were selected. These samples were powdered, mixed with potassium bromide, pelletized and taken up for FTIR analysis. The wavelength patterns were scrutinized by comparing with the peaks obtained by the reference standards of cystine. Spectra were also obtained from pure cystine. Comparison of spectra with those of whewellite and uric acid was performed. Then the samples were taken for Scanning electron microscopy with elemental distribution analysis X-ray (SEM–EDAX). The samples were made conductive by gold sputtering and were fed into JEOL JSM 35 C SEM machine. Morphology was recorded by taking photographs. Further elemental distribution analysis (EDAX) was carried out to identify the elemental composition. Of the 30 samples taken up for FTIR analysis, all showed spectra identifiable with the reference peaks for cystine. However, when these peaks were compared with those of whewellite and uric acid, all the stone samples showed duplication of peaks for whewellite and uric acid and whewellite. The pure cystine spectra showed identifiable peaks are in the range of 3026, 1618.28, 1485, 846.75 cm^{-1}, etc. (from the standard spectrum of pure cystine). All the analysis findings were correlated with EDAX findings. On doing EDAX, we could separately find out the components present in a mixture. Three stones contained elemental pattern to fit with those of cystine. Even though it is difficult to find out the presence of cystine molecule in FTIR, it is possible to recognize it through EDAX and will be possible to confirm the presence of cystine in mixed urinary stones.

Keywords Urinary stone · Cystine · FTIR · SEM · EDAX · Stone composition

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Y. M. Fazil Marickar (✉)
Department of Surgery, Zensa Hospital,
Trivandrum 695 009, India
e-mail: fazilmarickar@hotmail.com

P. R. Lekshmi · L. Varma · P. Koshy
Department of SEM, National Institute for Interdisciplinary Science and Technology (NIIST), Trivandrum 695019, India

Introduction

Cystine as a chemical constituent of urinary tract was first discovered by Wollaston in 1810 [1]. Stroh Meyer provided the classic microscopic description of hexagonal plate like crystals in 1824 [2]. Because of the absence of oxide, Berzelius in 1993 renamed the compound as cystine [3]. Cystine kidney stone due to cystinuria [4], an inherited disorder of the transport of amino acid results in an excess of cystine in urine and the formation of cystine stones. Literature survey reveals that cystine stones are very uncommon [5].

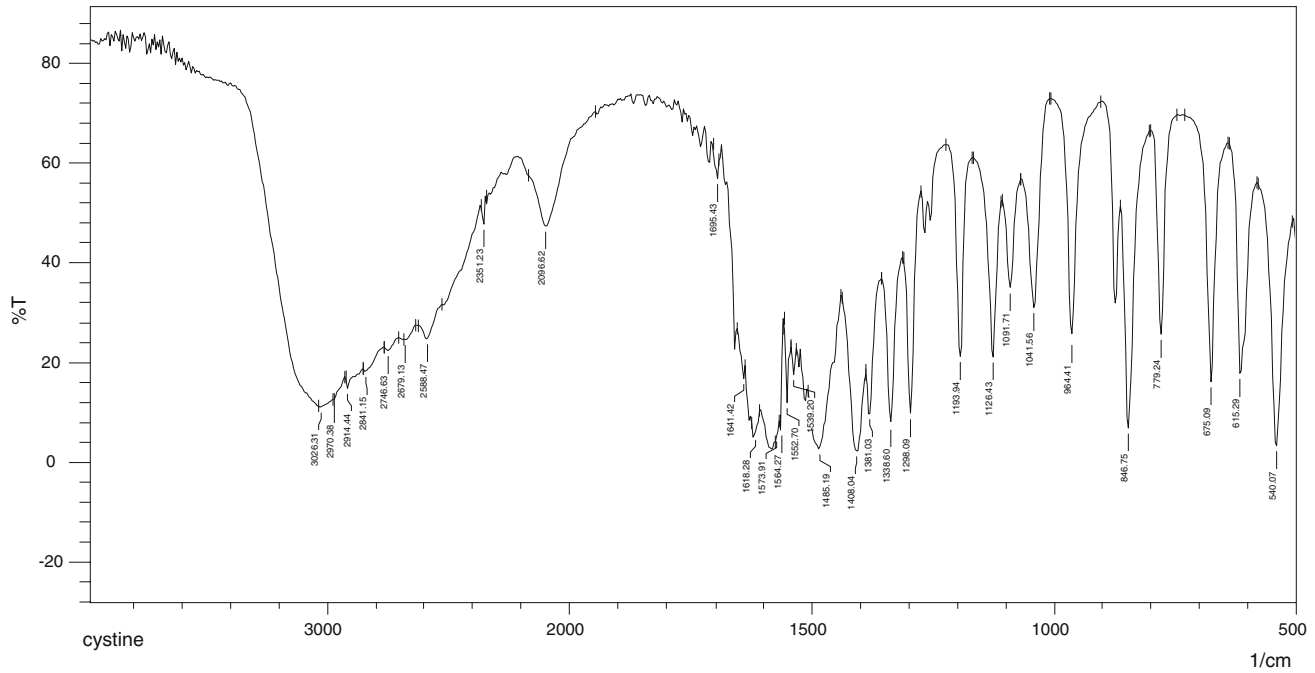

Fig. 1 FTIR spectrum of Sample 1

Proper analysis of stones is necessary for proper diagnosis and thus for proper treatment. Morphologic examination with Fourier Transform Infrared (FTIR) spectroscopy is of decisive interest for the rare but severe inherited or acquired stone diseases [6]. The routine, easy, and rapid measurements by FTIR give unambiguous information about the stone composition [7]. Modern scientific techniques like Raman Spectroscopy [8], X-ray diffraction [9], and Scanning electron microscopy with elemental distribution analysis X-ray (SEM–EDAX) [10] are very much important in stone analysis. The problem of analyzing cystine as a component in a mixture of stones using FTIR is significant. EDAX [11] makes it possible to get an idea about the percentage composition of each element present in calculi. Though it is difficult to identify cystine molecules in a mixed stone using FTIR analysis, EDAX makes it possible. The present paper describes the problems we encountered in analyzing cystine stones using FTIR spectroscopy and discuss its solutions.

Materials and methods

Chemical analysis

Out of 1,300 urinary stones analysed by ordinary wet chemical methods and infrared spectroscopy, 30 samples were reported to have cystine peaks in significant numbers and were selected.

FTIR analysis

Thirty stones were washed and dried. These stone samples were powdered, mixed with potassium bromide, and taken for FTIR analysis. Synthetic samples like pure cystine (purchased from SISCO Research Laboratories PVT. Ltd.), mixture of cystine, whewellite and uric acid, and mixture of whewellite and uric acid were also prepared according to the chemical report and their FTIR analyses were carried out. These reference spectra were compared with the spectra of stone samples.

SEM–EDAX analysis

The samples were taken for SEM–EDAX. The samples were made conductive by gold sputtering and were fed into JEOL JSM 35 C SEM machine. Morphology of the stone samples was recorded by taking photographs at different magnifications. Further elemental distribution analysis was carried out to identify the elemental composition.

Results and discussion

Among 1,300 stones collected, 30 were reported to have cystine in significant amounts and were selected for further investigation. Since cystine had many peaks, it was difficult to characterize using FTIR analysis. In order to clarify the FTIR result, morphological and elemental studies using

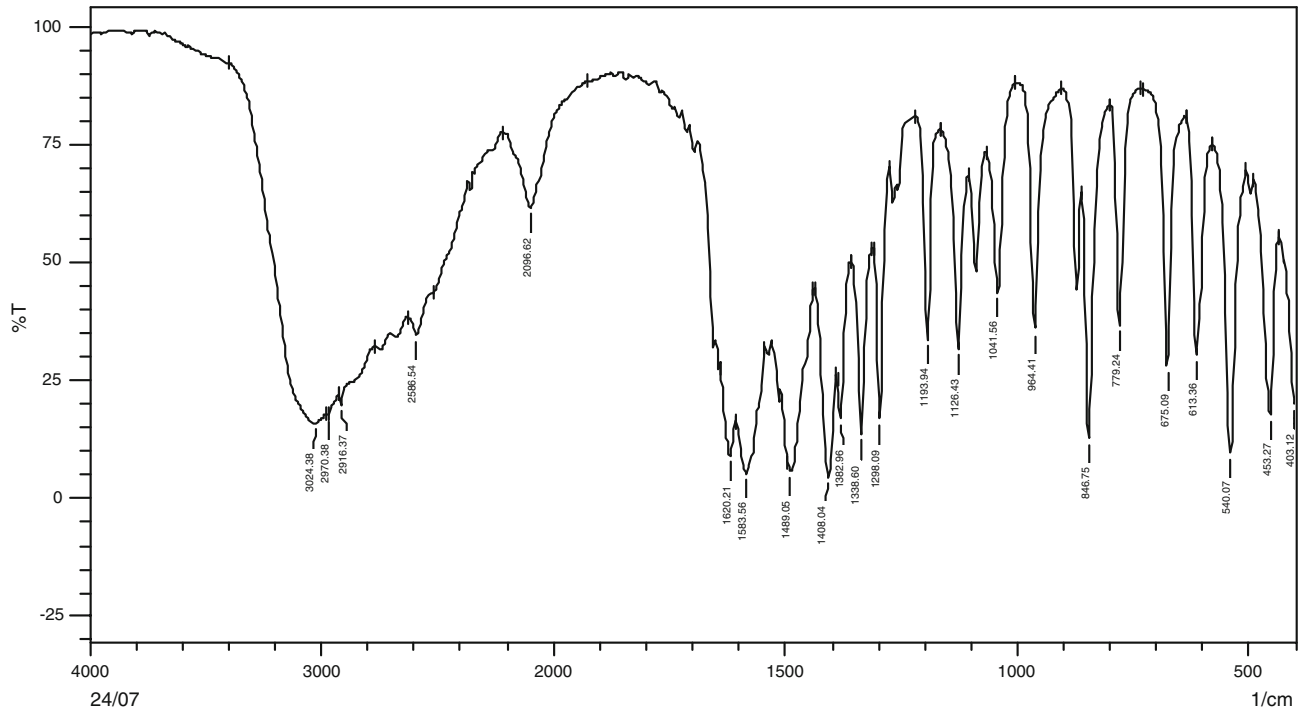

Fig. 2 FTIR spectrum of pure cystine

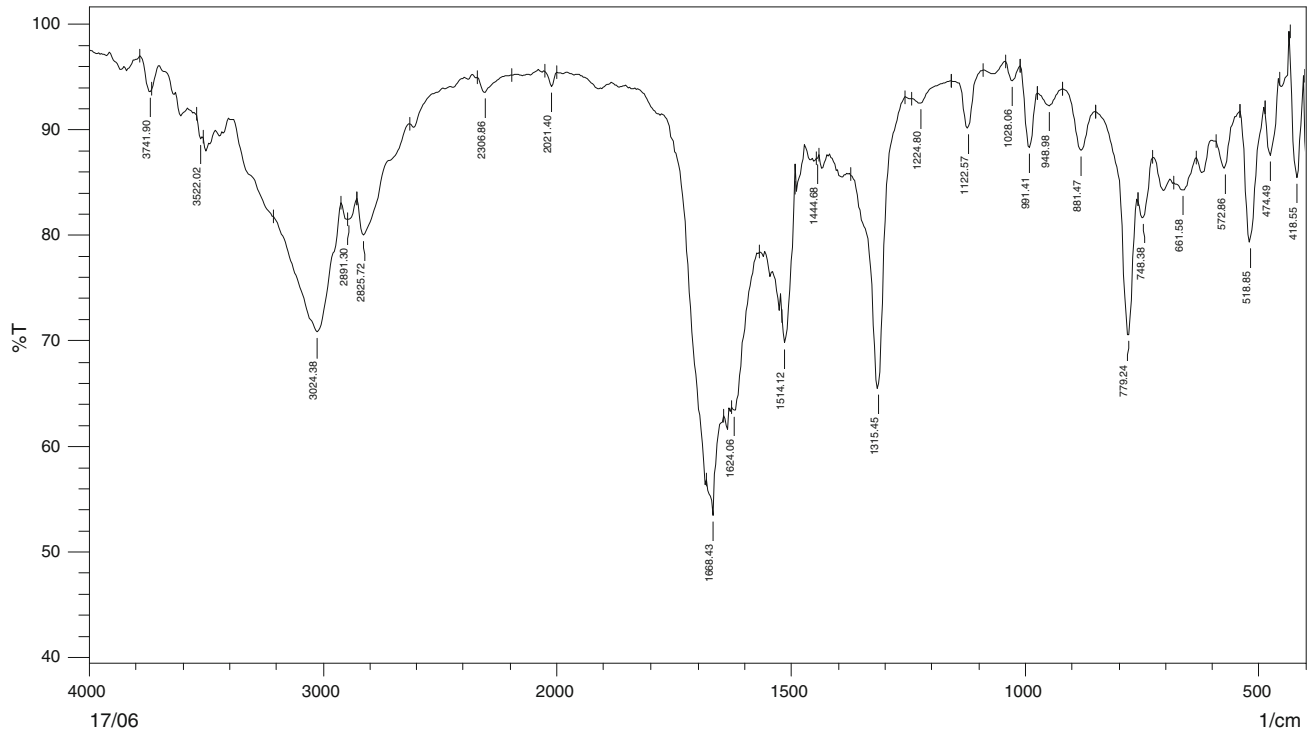

Fig. 3 FTIR spectrum of Sample 2

SEM–EDAX were also done. Prominent peak of sulfur in EDAX indicated the presence of cystine.

FTIR analysis of a few stone samples and their comparison with the reference spectra are detailed. A calculus collected from the stone clinic was initially reported as mixed stone containing uric acid and cystine (Fig. 1), but the same stone was confirmed as cystine alone as it produced a super imposable FTIR spectrum (Fig. 2) with that of pure cystine.

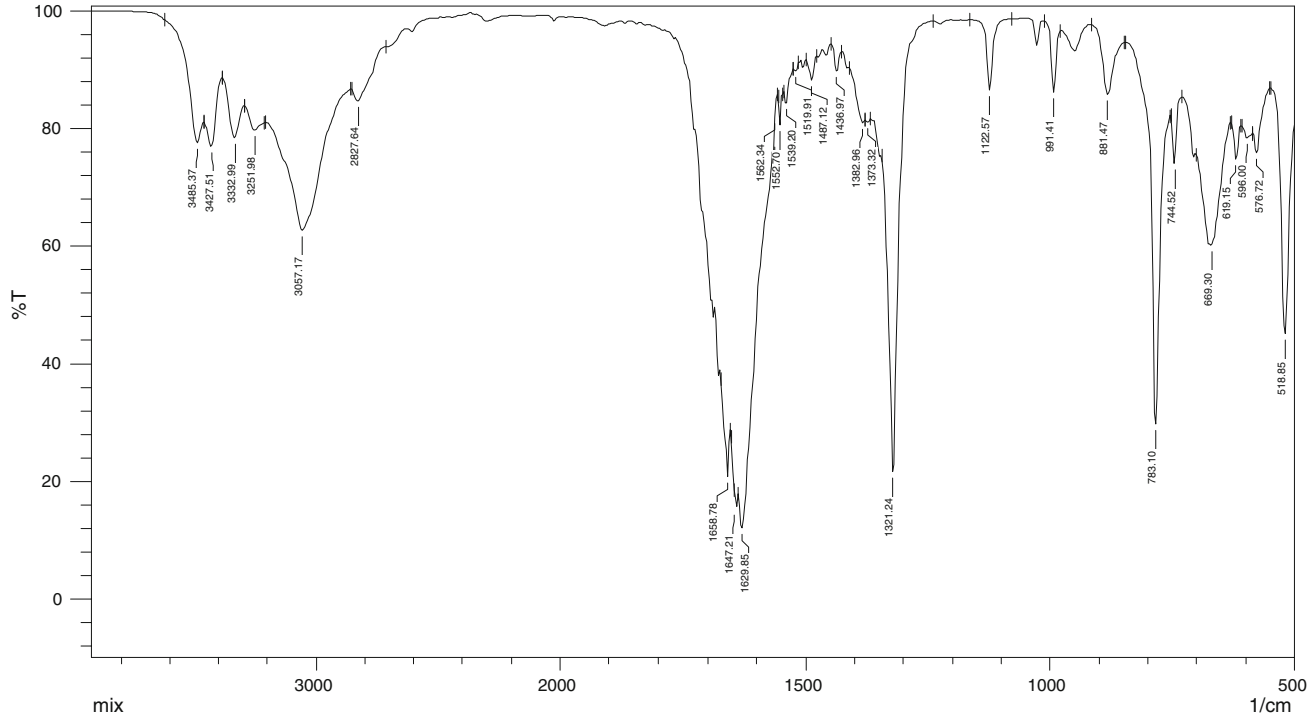

Fig. 4 FTIR spectrum of mixture of calcium oxalate monohydrate and uric acid in the ratio 4:1

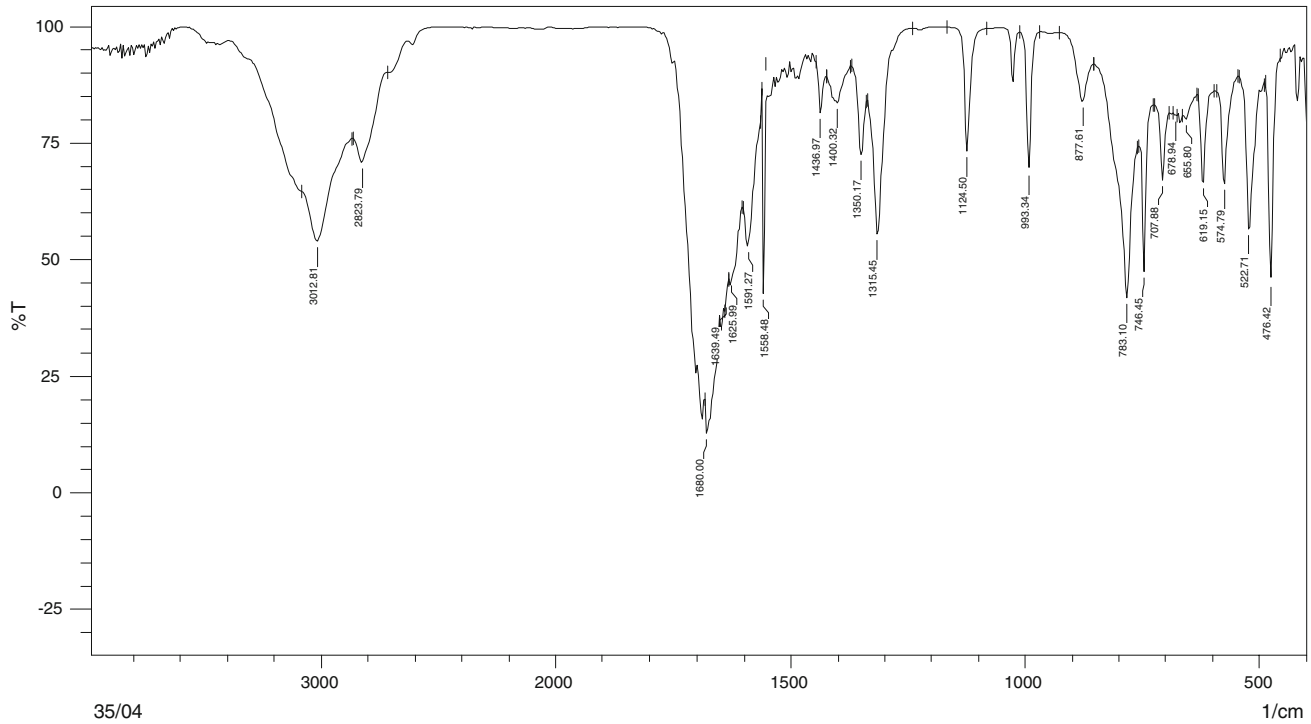

Fig. 5 FTIR spectrum of Sample 3

Stone sample 2 (Fig. 3) from urinary stone clinic was reported as a mixture of calcium oxalate monohydrate, uric acid, and cystine; but by analyzing synthetic mixture of calcium oxalate monohydrate and uric acid using FTIR (Fig. 4) and by comparing with the stone sample 2, it is evident that the sample does not have any trace of cystine.

Stone sample 3 (Fig. 5) from urinary stone clinic was reported as a mixture of calcium oxalate monohydrate, uric

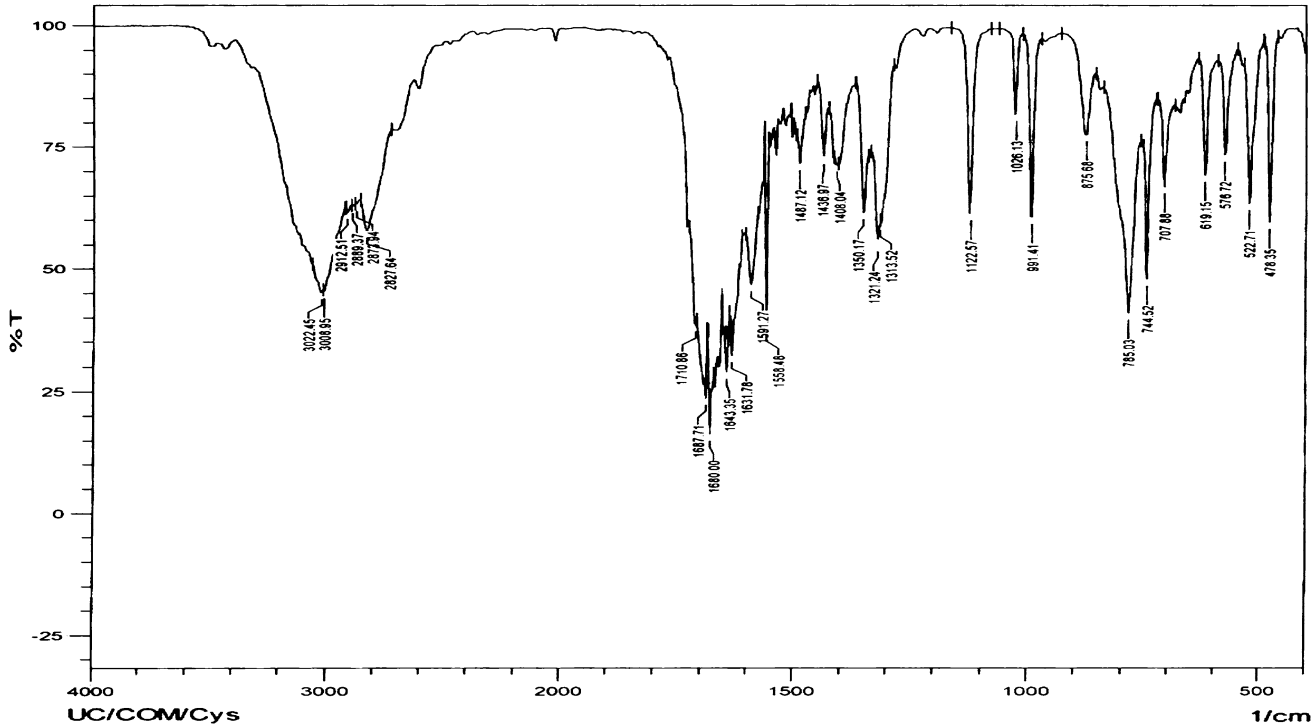

Fig. 6 FTIR spectrum of pure uric acid

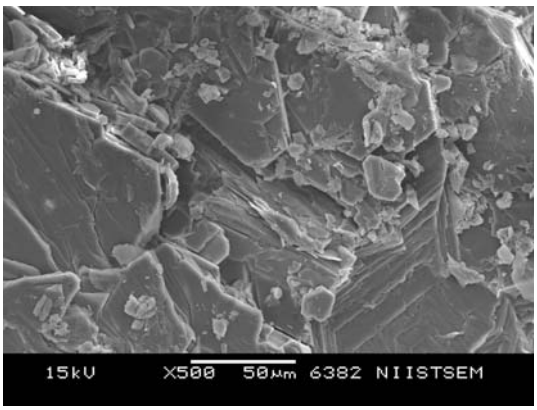

Fig. 7 SEM of stone sample 1

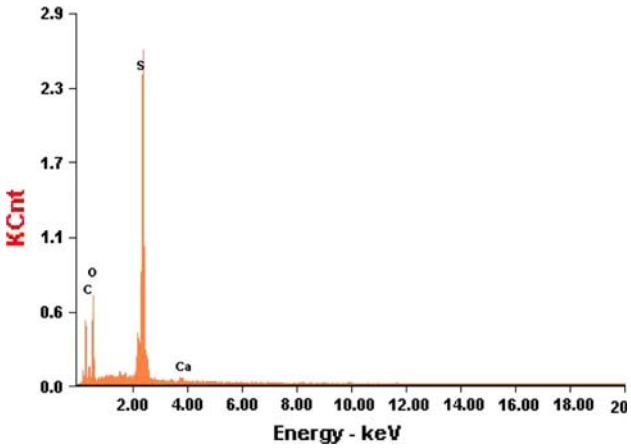

Fig. 8 EDAX of stone sample 1

acid and cystine. From our FTIR analysis of synthetic uric acid, it is evident that the sample 3 contained uric acid alone (Fig. 6).

Literature survey [12, 13] gives characteristic peaks assigned for cystine in the finger print region at 454, 541, 614, 676, 779, 847, 875, 964, 1041, 1091, 1127, 1193, 1257, 1297, 1337, 1381, 1408, 1487, 1584 and 1622, for calcium oxalate at 604, 660, 780, 1381 and 1618 and for uric acid at 621, 787, 1124 and 1589. The presence of these peaks in mixed stone containing all three components makes it difficult for an accurate analysis using FTIR spectroscope alone.

In order to overcome this difficulty, morphological and elemental analysis of these samples were done using SEM–EDAX. Detailed FTIR analysis showed that sample 1 contains cystine alone. However, wet chemical analysis gave an anomalous result that it contained a mixture of calcium oxalate monohydrate and uric acid. FTIR result was confirmed using SEM–EDAX analysis (Figs. 7, 8, respectively), which showed a prominent peak of sulfur, which indicated the presence of cystine.

Detailed FTIR analysis showed sample 2 to be a mixture of calcium oxalate monohydrate and uric acid. But wet chemical analysis gave an anomalous result that the sample contains calcium oxalate monohydrate, uric acid, and cystine. FTIR result was confirmed using SEM–EDAX

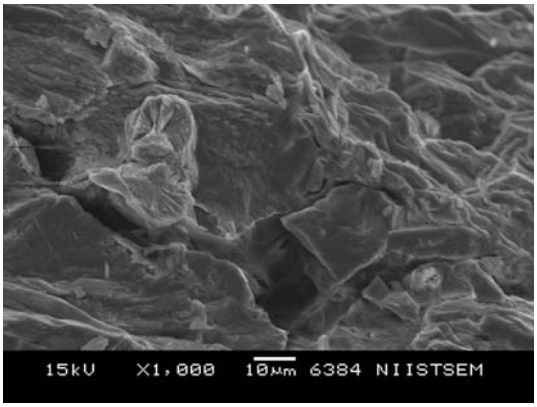

Fig. 9 SEM of stone sample 2

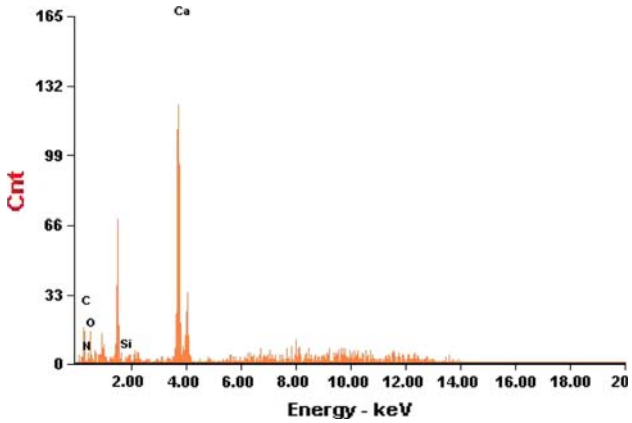

Fig. 10 EDAX of stone sample 2

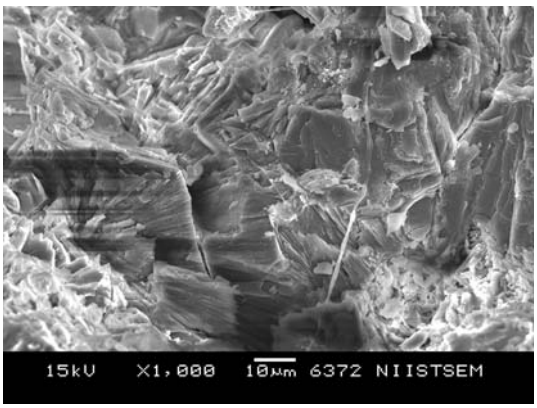

Fig. 11 SEM of stone sample 3

analysis (Figs. 9, 10, respectively) which showed prominent peaks of C, N, Ca, Si and O. Absence of sulfur peak indicated that there is no trace of cystine in this stone.

Detailed FTIR analysis showed sample 3 to be uric acid stone. But the wet chemical analysis of the same sample gave an anomalous result that the sample contains calcium oxalate monohydrate, uric acid, and cystine. FTIR result was confirmed using SEM–EDAX analysis (Figs. 11, 12,

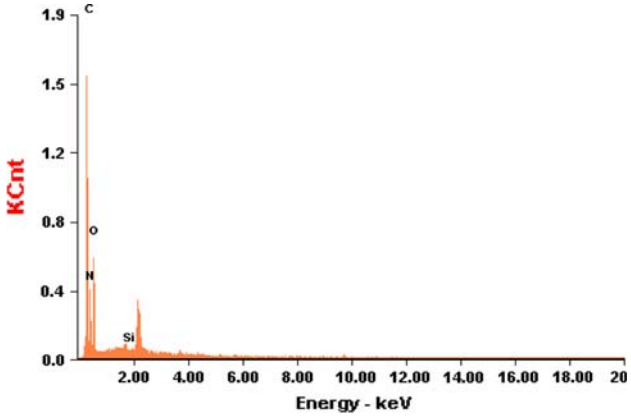

Fig. 12 EDAX of stone sample 3

respectively) which showed a prominent peak of nitrogen, which indicated the presence of uric acid.

From the above-illustrated examples, it is clear that FTIR results can be confirmed using EDAX results. The entire FTIR analysis findings were thus correlated with EDAX findings. On doing elemental distribution analysis, we could separately find out the components present in a mixture. It is clear that if a sample is of cystine composition then EDAX result will show a prominent peak for sulphur. On doing so, we found that among the 30 stone samples reported only three were of cystine composition.

Conclusion

To analyse cystine compounds in a mixture is quite difficult as it may show peaks having similarity with the mixture of calcium oxalate monohydrate and uric acid. Even though it is difficult to find out the presence of cystine molecule in FTIR, it is possible to recognize it through EDAX and will be possible to confirm the presence of cystine in mixed urinary stones.

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References

1. Wollaston WH (1810) On cystic oxide: a new species of urinary calulus. Trans R Soc Lond 100:223–230
2. Noehden GH (1824) Scientific notices-chemistry, cystic oxide-communicated in a letter from Dr.Noehden to Mr.Children. Ann Philos 7:146
3. Berzelius JJ (1833) Calculus urinaires. Tracte Chem 7:424
4. Their SO, Segal S (1972) Cystinuria. In: Stanbury JB, Wyangaarden JB, Fredrickson DS (eds) The metabolic basis of inherited diseases. McGraw-Hill, NewYork, pp 1504–1519

5. Kaohsiung YH (2007) Urinary stone analysis of 1000 patients in southern Taiwan-choie. J Med Sci 23(2):63–66
6. Daudon M, Jungers P (2004) Clinical value of crystalluria and quantitative morphoconstitutional analysis of urinary calculi. Nephron Physiol 98:31–36
7. Paluszkiewicz C, Sciesinski J, Galka M (1988) Analysis of renal stones by FT-IR spectroscopy. Mikrochim Acta 1:45–48
8. Ellis DI, Goodacre R (2006) Metabolic fingerprinting in disease diagnosis: biomedical application of infrared and Raman spectroscopy. Analyst 131:875–885
9. Bhatt AP, Parimal P (2008) Analysis of urinary stone constituents using powder X-ray diffraction and FTIR. J Chem Sci 120(2):267–273
10. Walther P, Wehrli E, Hermann R, Mller M (1995) Double layer coating for high resolution low temperature SEM. J Microsc 179:229–237
11. Lee KM, Cai Z, Griggs JA, Guiatas L, Lee DJ, Okabe T (2004) SEM/EDS evaluation of porcelain adherence to gold-coated cast titanium. J Biomed Mater Res B Appl Biomater 68(2):165–173
12. Murphy BT, Pyrah LN (1962) The composition, structure and mechanism of formation of urinary calculi. Br J Urol Lond 34:129–159
13. Estepa L, Daudon M (1997) Contribution of Fourier Transform Infrared Spectroscopy to the identification of urinary stones and kidney crystal deposits. Biospectroscopy 3:347–369