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SYMPOSIUM PAPER

EDAX versus FTIR in mixed stones

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Abstract Mixed stones form a significant number of all urinary stones. Accurate analysis of individual areas of stones is fraught with uncertainties. Scanning electron microscopy with elemental distribution analysis (SEM-EDAX) is a very important tool in assessing stone composition. The objective of this paper is to project the role of the combination of Fourier transform infrared (FTIR) spectroscopy and SEM-EDAX combination in achieving a total understanding of mixed stone morphology. Ten mixed urinary stones were washed and dried and the composition recognized by analysis of FTIR spectra by comparing with the spectra of pure components. Spectra for different layers were obtained. Then the stone samples were further studied by SEM-EDAX analysis. 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. As much as 80% of the stones studied were mixtures of calcium oxalate monohydrate (whewellite) and calcium phosphate (hydroxyapatite) in various proportions. Quantitative evaluation of components was achieved through FTIR and SEM-EDAX analysis. It was possible to get an idea about the spatial distribution of molecules using SEM analysis. The composition of different areas was identified using EDAX. Analyzing with EDAX, it was possible to obtain the percentage of different elements present in a single sample. The study concludes that the most common mixed stone encountered in the study is a mixture of calcium oxalate monohydrate and calcium phosphate in a definite proportion. The combination identified not only the molecular species present in the calculus, but also the crystalline forms within chemical constituents. Using EDAX, the amount of calcium, phosphorus, oxygen and carbon present in the stone sample could be well understood.

Keywords Urinary stone · Mixed stone · Composition · SEM · EDAX · FTIR · COM · Phosphate

Introduction

Urinary stones have become increasingly common in most parts of India. Urinary calculi are crystals with mainly one or more of the three components, oxalate, phosphate and uric acid [1]. Cystine stones are less common [2]. Some stones are made of single crystalline component, but many are mixtures of different components. A study of the chemical composition of renal stones is important for understanding their origin [3]. Infrared (IR) spectroscopy has been successfully applied to the analysis of urinary calculi for the last three decades [4, 5]. Scanning electron microscopy (SEM) is one of the most versatile instruments used for the morphological study of urinary calculi [6]. Elementary distribution analysis (EDAX) is used to get an idea of the percentage composition of each element present in a stone sample [7]. The morphological appearances and ultramicroscopic appearances can be accurately assessed to understand the genesis of stone. However, it is not feasible

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to perform this in routine clinical practice. Fourier transform infrared (FTIR) is more popular, but lacks details of morphology of individual areas of stones. A combination of these will give insight into the actual morphology of urinary stones. In this paper, we compare the usefulness of scanning electron microscopy with elementary distribution analysis (SEM-EDAX) with that of FTIR spectroscopy in analyzing mixed urinary stones.

Materials and methods

Ten mixed stones of minimum 10 mm diameter were collected from the stone clinic. The samples were washed, dried and fragmented. A total of 30 fragments were collected from the inner, mid and outer regions.

These fragments were pulverized using mortar and pestle. The powdered specimens were mixed with potassium bromide in the ratio of 1:20 and FTIR analysis was carried out. IR radiation was passed through the samples. The IR spectrum representing the molecular absorption and transmission created a molecular fingerprint of the sample. The IR spectra obtained were recorded for the different layers of the stone. These spectral data were compared with those of pure synthetic components and the spectral peaks were identified. The rest of the stone fragment was taken up for SEM-EDAX. Fragments less than 1 cm in size were gold sputtered to make them conductive.

The samples were further studied by SEM-EDAX. For making the samples conductive, gold sputtering was done. A sputter coater was used to coat the urinary stone fragments with a thin layer of gold to 100 Å. This made them conducive to be viewed by the Jeol JSM 35C SEM machine. Electron beams were allowed to fall on the sample and the secondary electrons from the samples were detected. These were then analyzed using SEM-EDAX.

Results

In the 30 fragments of 10 stone samples anlyzed by FTIR and SEM-EDAX, 80% of stone composition was identified as a mixture of calcium oxalate monohydrate (whewellite) and calcium phosphate (hydroxyapatite) in various proportions. The rest of the samples were identified as mixtures of calcium oxalate dihydrate (weddellite) and calcium phosphate. The core portions of the calculi showed more amount of calcium and phosphorus than the outer regions in the EDAX analysis.

Fourier transform infrared spectrum of the outer porous portion of the sample (Fig. 1) shows peaks at 1,315 and 1,610. These were attributed to asymmetric and symmetric stretching of oxalate anion showing that the sample contains whewellite. The medium band centered at 1,036 indicates the presence of phosphate due to v_3 asymmetric stretching vibration. Peaks at 950 and 885 were assigned to O–H deformation and confirm the presence of whewellite. Peaks at 781, 664 and 518 were due to vibrations of the O–C–O group in whewellite. Peaks in the region 2,700–3,800 were due to O–H stretching vibration of crystallized water in calcium oxalate and calcium phosphate. Hence, the FTIR spectrum shows the outer portion of the sample to be a mixture of calcium oxalate monohydrate (whewellite) and calcium phosphate.

Fourier transform infrared spectrum of the inner hard portion of the sample (Fig. 2) shows peaks at 1,324 and 1,631 that were assigned to asymmetric and symmetric stretching of oxalate anion in weddellite. Peaks at 1,004, 1,034 and 1,133 were due to v_3 asymmetric stretching vibration of phosphate in calcium phosphate. Very weak peak at 1,432, due to HNH deformation of NH_4,indicates the presence of struvite. A sharp peak at 916, attributed to O–H deformation, confirms the presence of weddellite. Bands at 770,600 and 520 were assigned to vibrations of the O–C–O group in weddellite. Peaks in the region of 2,700–3,800 are due to O–H stretching vibration of crystallized water in calcium oxalate, calcium phosphate and struvite. Thus, the FTIR spectrum shows the inner hard portion of the calculi sample to be a mixture of minerals: calcium oxalate dihydrate (weddellite), calcium phosphate and struvite.

The stone fragment was then taken up for SEM-EDAX. It was possible to get an idea of the spatial distribution of molecules using SEM analysis. The amount of elements, especially calcium, phosphorus, oxygen and carbon, present in the stone sample was well understood from EDAX. Analyzing with EDAX, it was possible to obtain the percentage of different elements present in a single sample.

After the analysis of the morphology of stone samples, EDAX probe was pointed at different areas and elemental analysis of the samples at different regions was carried out. Ca, C, P and O were the main elements identified. From EDAX analysis, the presence of calcium, phosphorus and oxygen revealed that the sample was of a mixed composition of both calcium oxalate and calcium phosphate. Also, it clearly showed that the outer region (Fig. 3) contained less amount of calcium and phosphorus compared to the inner region (Fig. 4).

Hence, FTIR analysis confirmed the SEM-EDAX analysis. All the ten samples showed a mixed composition in all the different layers taken.

Discussion

Urolithiasis is a chronic, debilitating disease that causes significant morbidity. It is a necessity to study the actual

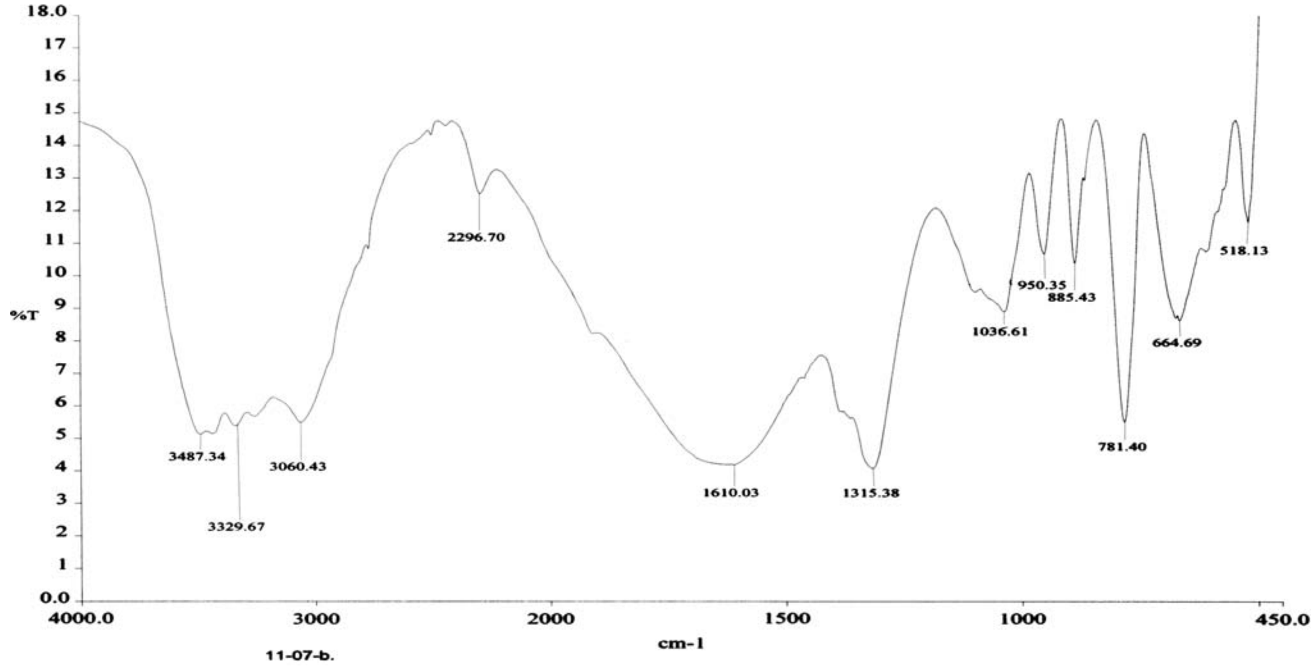


Fig. 1 FTIR analysis spectrum showing the outer porous portion of a sample

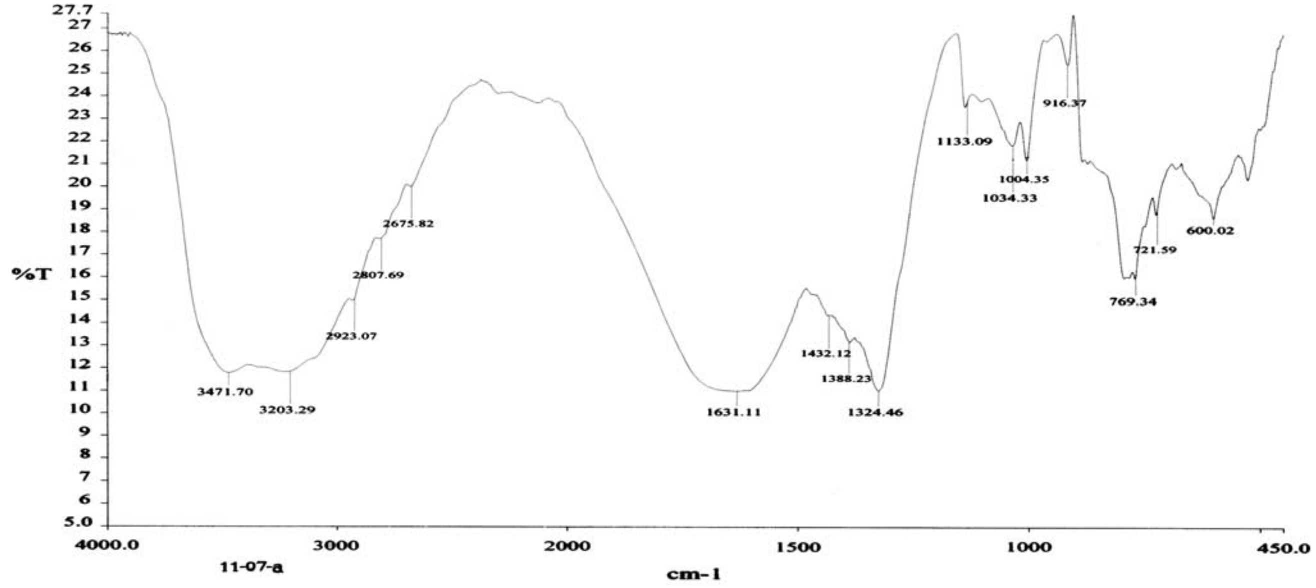
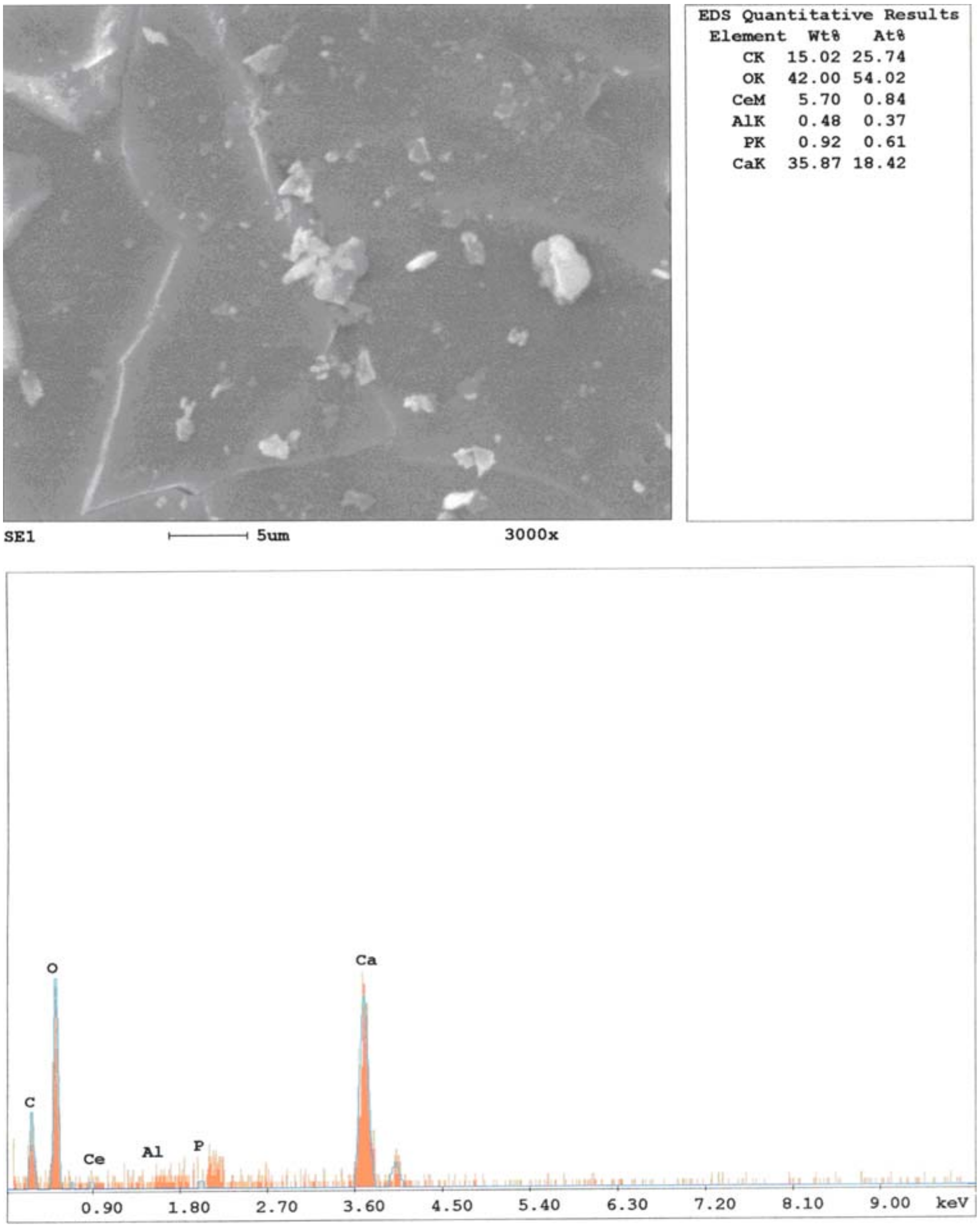


Fig. 2 FTIR analysis spectrum showing the inner hard portion of a sample

pathology of stone formation, so that the effect of changes produced by the environment and calculogenic diet on the process of stone formation can be understood in order to make adequate preventive measures.

Stone components may be mineral, organic or both. More than 65 different molecules (including 25% of exogenous origin) have been found in urinary calculi. The same chemical components may crystallize in different forms. Proper stone analysis has to identify not only the molecular species present in the calculus, but also the crystalline forms within chemical constituents [8]. Most stones are of mixed composition. Among heterogeneous calculi, about 80% are made of a mixture of calcium oxalate monohydrate and calcium phosphate in various proportions. Quantitative evaluation of components is needed to provide full information. This can be achieved through FTIR and SEM-EDAX analysis [9, 10].

FT-IR analysis is done by passing IR radiation through the sample and producing a molecular fingerprint. Like a fingerprint, no two unique molecular structures produce the



EDS Quantitative Results

Element	Wt%	At%
CK	15.02	25.74
OK	42.00	54.02
CeM	5.70	0.84
AlK	0.48	0.37
PK	0.92	0.61
CaK	35.87	18.42

Fig. 3 SEM-EDAX showing the outer porous region of a sample

same IR spectrum [11]. This makes IR spectroscopy useful for several types of analysis [12].

Scanning electron microscope uses electrons rather than light to form an image [13]. It has a large depth of field, which allows a large amount of the sample to be focused at a time. SEM produces images of high resolution, which means that closely spaced features can be examined at a high magnification [14]. Preparation of sample is relatively easy since most SEMs only require the sample to be conductive [15]. The spatial distribution of major and trace elements can be studied in a range of human kidney and bladder stones with well-documented histories to understand their initiation and formation.

Currently, many physical and chemical methods are available for urinary stone analysis. No single method is sufficient to provide all the clinically useful information on the structure and composition of the stones. A combination of refined morphological and structural examination of stone with optical microscopy, complemented by compositional analysis using IR spectroscopy of core, cross section and surface of calculi, provides a precise and reliable method for identifying the structure and crystalline composition, and permits quantification of stone components while being highly cost-effective.

Fourier transform infrared analysis is more modern, less time consuming and more accurate. SEM-EDAX will give a clear indication of the structure of the stone on the surface and interior. But this investigation is more expensive and cumbersome and cannot be utilized for routine analysis of stones. No single method is sufficient to provide all the

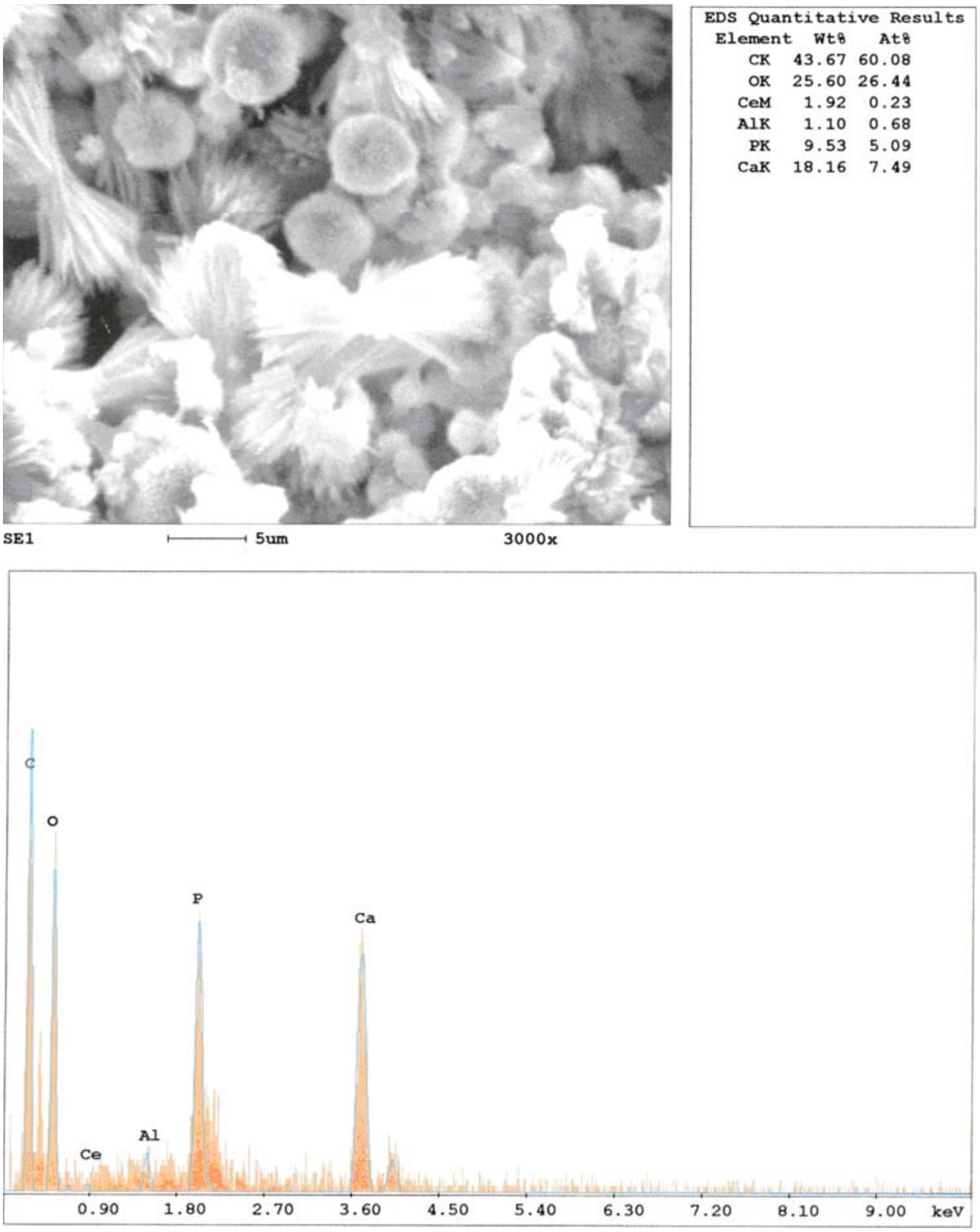


Fig. 4 SEM-EDAX showing the inner core region of a sample

EDS Quantitative Results

Element	Wt%	At%
CK	43.67	60.08
OK	25.60	26.44
CeM	1.92	0.23
AlK	1.10	0.68
PK	9.53	5.09
CaK	18.16	7.49

clinical information on the structure and composition of urinary stones. Correlating the findings of SEM-EDAX with FTIR spectral peaks, we can generate more meaningful data on a complete analysis of urinary stones.

It is hoped that the study will throw light on the actual pathology of stone formation, which would ultimately help in charting out preventive measures for stone formation.

Conclusion

The most common mixed stone encountered in the study is a mixture of calcium oxalate monohydrate and calcium phosphate in a definite proportion. The combination identified not only the molecular species present in the calculus, but also the crystalline forms within chemical constituents. Using EDAX, the amount of calcium, phosphorus, oxygen and carbon present in the stone sample could be well understood.

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