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SYMPOSIUM PAPER

Elemental distribution analysis of urinary crystals

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Abstract Various crystals are seen in human urine. Some of them, particularly calcium oxalate dihydrate, are seen normally. Pathological crystals indicate crystal formation initiating urinary stones. Unfortunately, many of the relevant crystals are not recognized in light microscopic analysis of the urinary deposit performed in most of the clinical laboratories. Many crystals are not clearly identifiable under the ordinary light microscopy. The objective of the present study was to perform scanning electron microscopic (SEM) assessment of various urinary deposits and confirm the identity by elemental distribution analysis (EDAX). 50 samples of urinary deposits were collected from urinary stone clinic. Deposits containing significant crystalluria (more than 10 per HPF) were collected under liquid paraffin in special containers and taken up for SEM studies. The deposited crystals were retrieved with appropriate Pasteur pipettes, and placed on micropore filter paper discs. The fluid was absorbed by thicker layers of filter paper underneath and discs were fixed to brass studs. They were then gold sputtered to 100 Å and examined under SEM (Jeol JSM 35C microscope). When crystals were seen, their morphology was recorded by taking photographs at different angles. At appropriate magnification, EDAX probe was pointed to the crystals under study and the wave patterns analyzed. Components of the crystals were recognized by utilizing the data. All the samples analyzed contained significant number of crystals. All samples contained more than one type of crystal. The commonest crystals encountered included calcium oxalate monohydrate (whewellite 22%), calcium oxalate dihydrate (weddellite 32%), uric acid (10%), calcium phosphates, namely, apatite (4%), brushite (6%), struvite (6%) and octocalcium phosphate (2%). The morphological appearances of urinary crystals described were correlated with the wavelengths obtained through elemental distribution analysis. Various urinary crystals that are not reported under light microscopy could be recognized by SEM–EDAX combination. EDAX is a significant tool for recognizing unknown crystals not identified by ordinary light microscopy or SEM alone.

Keywords Urine · EDAX · Whewellite · Weddellite · Apatite · Brushite · Struvite · Uric acid

Introduction

Various crystals are seen in human urine. Oxalate, phosphate, uric acid and urate crystals are generally seen in urinary calculi [1]. Urinary deposits may also contain magnesium ammonium phosphate, carbonate apatite and mono ammonium urate [2]. Concentration of these crystals leads to the formation of urinary calculi. Calcium stones are most common, comprising 75% of all urinary calculi. They may be pure stones of calcium oxalate or calcium phosphate or a mixture of calcium oxalate and calcium phosphate [3]. Many stones are not homogeneous. Some have a

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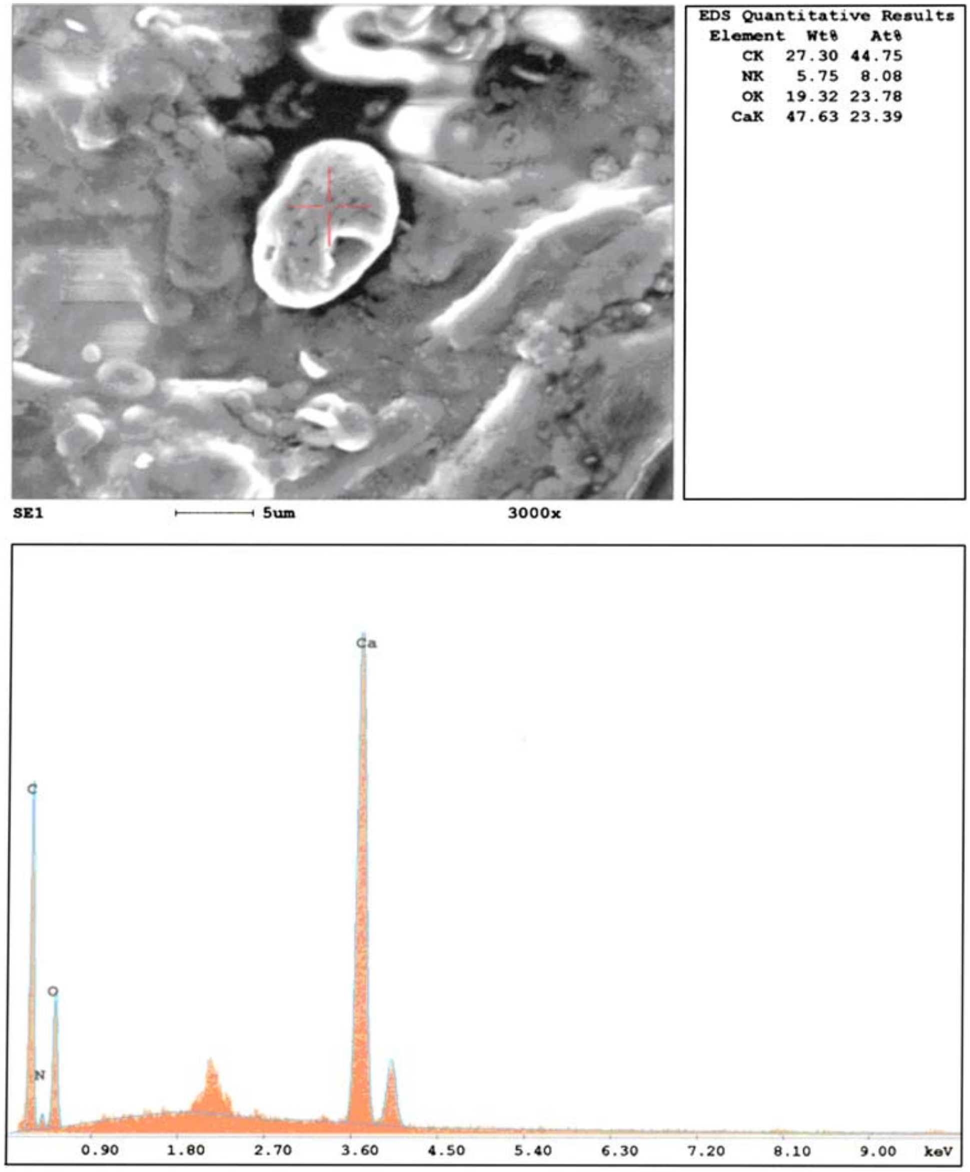


Fig. 1 Calcium oxalatemonohydrate crystal: **a** SEM, **b** EDAX

nucleus of different composition from the surrounding matrix.

Crystal formation can be caused due to several reasons [4]. In the case of insufficient intake of water or due to the decreased rate of excretion, the crystallogenic substances in the urine can be concentrated, leading to crystal formation. A change in pH value in the urine can lead to the crystal growth. The decrease in solubility of crystallogenic substances in urine can also lead to the formation of crystals.

The morphology, composition, etc., of crystal deposits can be evaluated using optical microscopy and scanning electron microscopy (SEM) together with elemental distribution analysis [5]. Unfortunately many of the relevant crystals are not recognized in ordinary microscopic analysis of urinary deposits performed in most of the clinical laboratories. This is because the light waves have a limited wavelength compared with that of electrons. Hence it is possible to identify the crystal, however small it may be, through SEM [6]. SEM can produce very high-resolution images of a sample surface, revealing details about 1–5 nm in size [7].

The use of optical microscopy and SEM combined permits a more far-reaching investigation in modern research areas [8]. The subsequent elemental distribution analysis (EDAX system) allows the determination of mixed phases in a single crystal. The instrument combination was successfully tried on urinary crystals and thin sections of urinary calculi. The description of some findings, with the aid of examples, explains the efficiency of the system in the expansion of urinary calculus analysis.

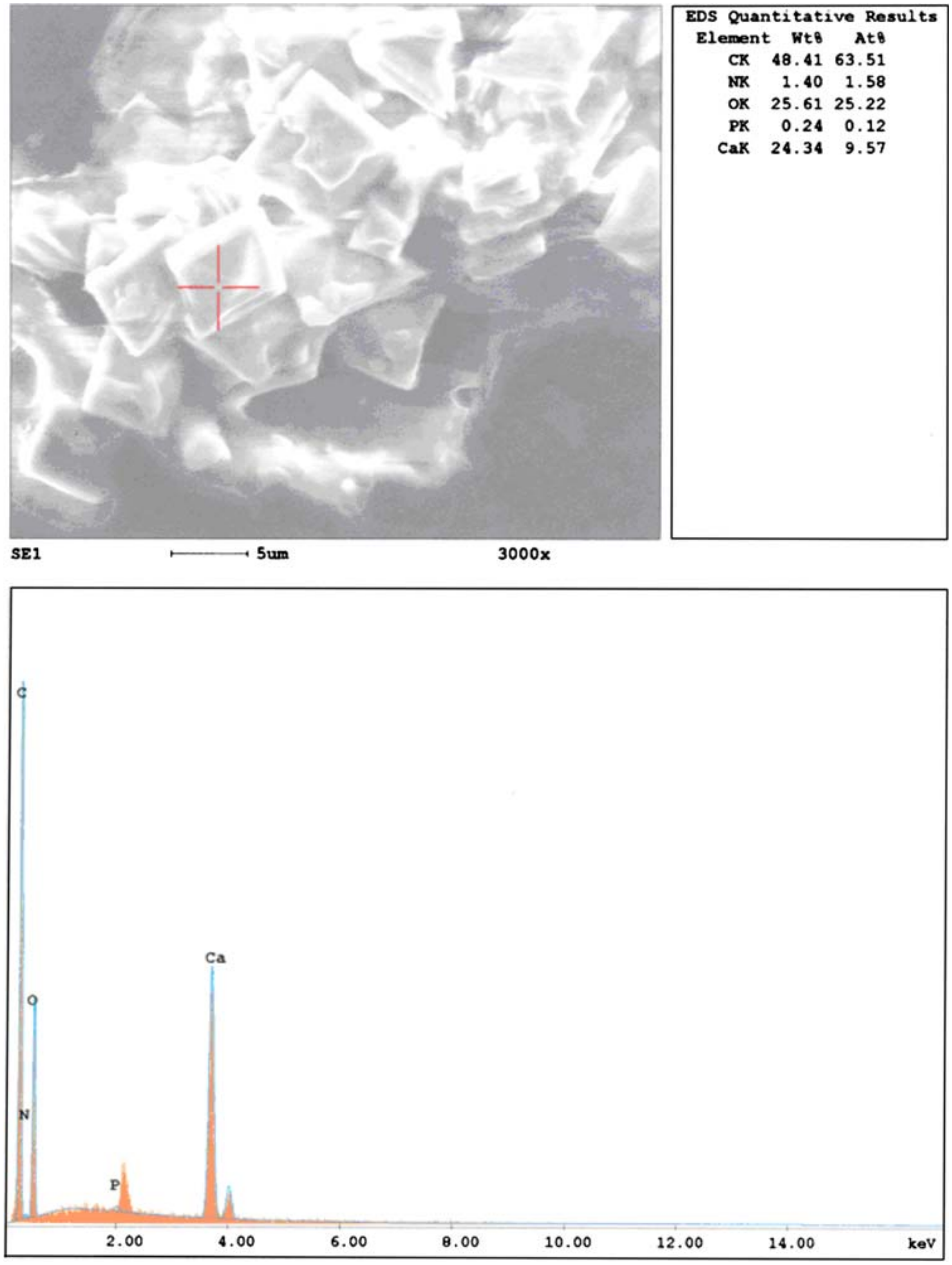


Fig. 2 Calcium oxalate dihydrate crystal: **a** SEM, **b** EDAX

Materials and methods

Urinary deposits from 50 samples were collected from urinary stone clinic. Deposits containing significant crystals (more than 10 per HPF) were collected under liquid paraffin in special containers. The deposited crystals were retrieved with appropriate Pasteur pipettes, and placed on micropore filter paper discs. Thicker layers of filter paper absorbed the paraffin fluid. The dry filter papers along with invisible crystals were fixed to brass studs. These brass studs were taken up for gold sputtering, in order to make them conductive, using a sputter coater [9]. They were gold sputtered to 100 Å and examined under SEM (JEOL JSM 35 C microscope). In-depth analysis of crystals was possible using SEM as electrons were allowed to fall on the sample. When crystals were seen, their morphology was recorded by taking photographs at different angles. SEM could give a maximum magnification of 50,000 times. Photographs were taken at different magnifications (30–5,000) and were recorded. At appropriate magnification, EDAX probe was pointed to the crystals under study and the wave patterns were analyzed. Components of the crystals were recognized and analyzed utilizing these data.

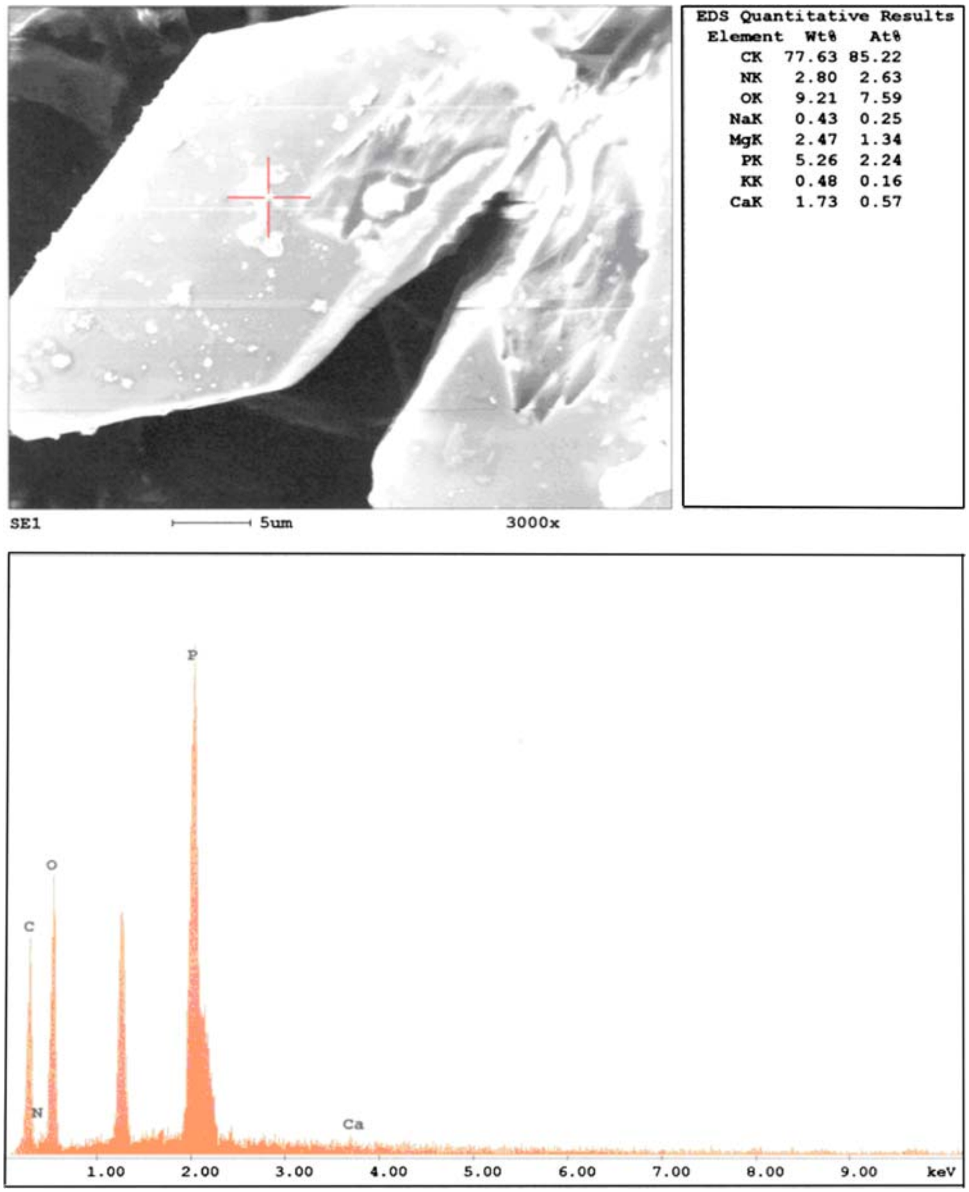


Fig. 3 Brusite crystal: **a** SEM, **b** EDAX

Results and discussion

The 50 samples of urinary deposits analyzed contained significant number of crystals. All the samples contained more than one type of crystal. The commonest crystals encountered included calcium oxalate monohydrate (whewellite), 36%; calcium oxalate dihydrate (weddellite), 44%; calcium phosphates (hydroxyapatite, 4%; brushite, 6%; struvite, 6%; octocalcium phosphate, 2%); and aluminum–silicon deposits, 2%.

Calcium oxalate crystals Calcium oxalate (calcium oxalate monohydrate and calcium oxalate dihydrate) is probably the crystal that one meets the most frequently in urinary sediment. In some cases, the crystallization of the calcium oxalate is massive and disastrous. Fatty acids compete with oxalate for the intestinal calcium. Morphological analysis using SEM studies showed that calcium oxalate monohydrate (Fig. 1) crystals appeared in spherical shape and calcium oxalate dihydrate (Fig. 2) appeared in rectangular pyramidal shape. EDAX results show the presence of Ca, O and C as predominant elements. That gives support to the SEM result.

Brushite Calcium hydrogen phosphate dihydrate (CHPD) or brushite crystals are well-known urinary crystals and frequently found in urinary stones. Morphological analysis using SEM gives grown crystals having plate like and star shaped crystals (Fig. 3) of brushite. From EDAX analysis, predominant element present was found to be phosphorus.

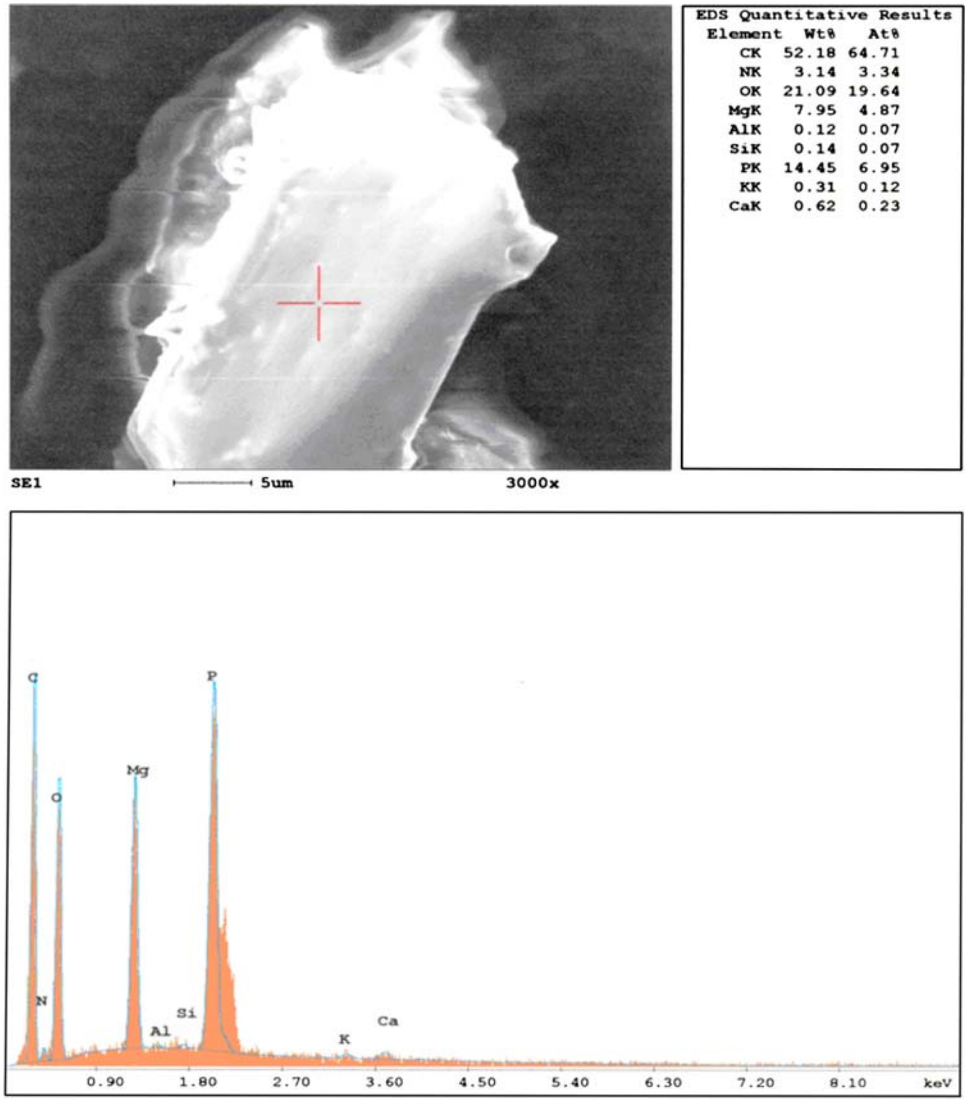


Fig. 4 Struvite crystal: **a** SEM, **b** EDAX

Presence of other elements like Ca, C and O confirms the SEM result.

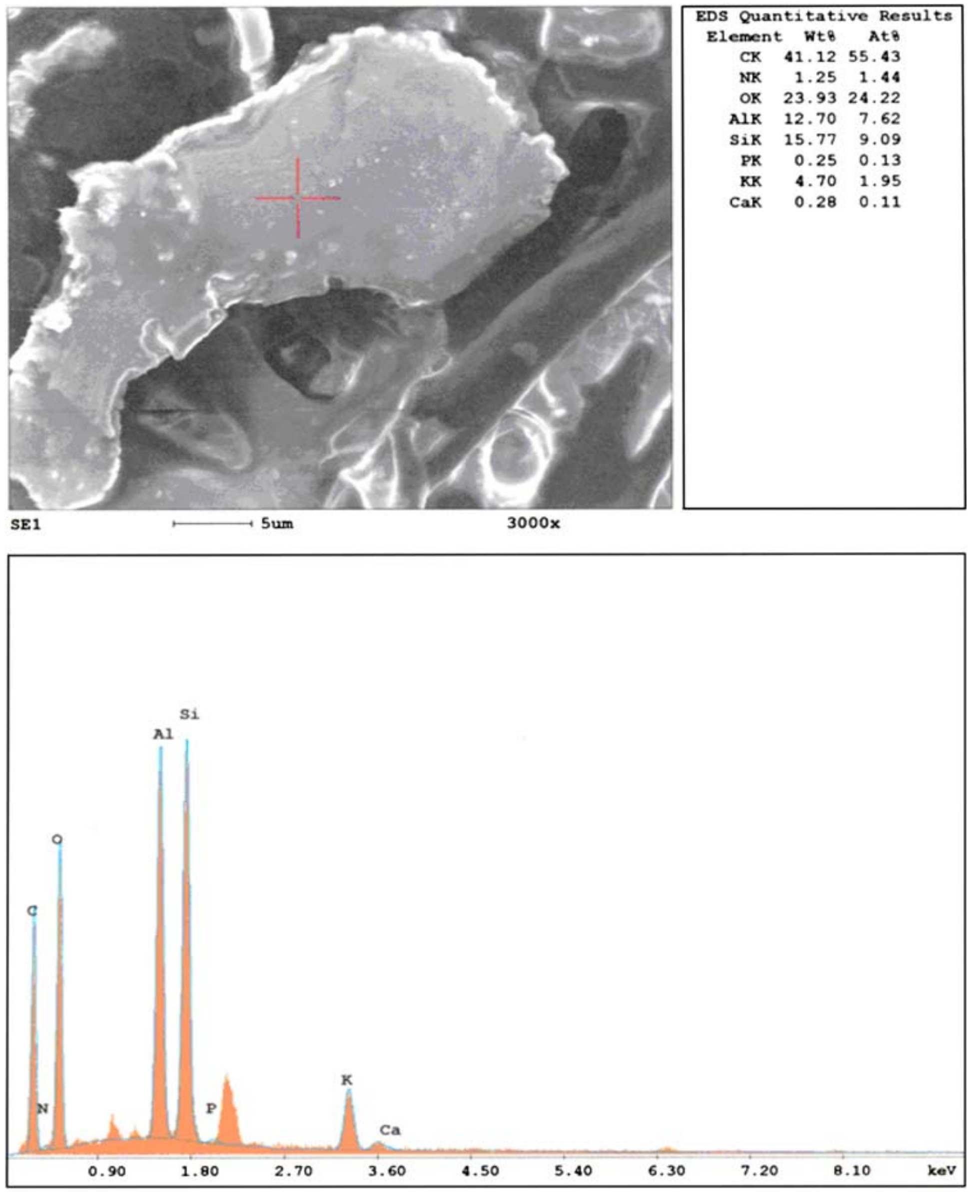
Struvite [10] is the name given to the crystal composed of magnesium, ammonium and phosphate. Struvite crystals are not unusual in normal urine and are usually of no consequence but when they are present in very large amounts, they can form stones. Morphological analysis using SEM (Fig. 4) showed developed crystals having rectangular platelet and prismatic shaped crystals. EDAX results indicate presence of magnesium, phosphorus and nitrogen confirming the SEM result.

Aluminum and silicon deposits Among the samples we collected, we came across a rare result. Our analysis pointed out that one among the sample contained a rare composition containing aluminum and silicon (Fig. 5).

Conclusion

Various urinary crystals that are not reported under optical microscopy could be recognized by SEM–EDAX combination. Microscopic crystals were found by SEM analysis in urine of stone forming patients as well as in urines of non-stone formers. Using scanning electron microscopy with an appropriate magnification, we can study the morphology and the crystal structure of various deposits and the result can be further used as references. Elemental distribution analysis makes it possible to confirm the SEM results and also percentage of different elements present in a single sample can be evaluated. So it is now possible to correlate the results of every analysis with the appropriate diagnosis and therapeutic regimen.

Fig. 5 Aluminum and silicon deposits: **a** SEM **b** EDAX



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