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The role of SEM and infrared spectroscopy techniques in identification of the mineralogy of kidney stones: case study from Fallujah City, Western Iraq

Hassan A.A. Al-Jumaily , Omar A.M. Mohammad , Anwaar S.J. Al-Maadhidi

Department of applied geology , college of science , Kirkuk University , Kirkuk , Iraq

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Corresponding Author:
Name: Omar A.M. Mohammad
E-mail: omarm@uokirkuk.edu.iq
Tel:

ABSTRACT

The current study is concerned with the mineralogical aspects of kidney stones from urolithiasis patients of different ages and genders in Fallujah city hospital. The following techniques were implemented as follows; X - ray diffraction (XRD) used to recognize the mineral components of the studied kidney stones, Infrared Spectroscopy (IR) to discover the type of chemical compounds of the studied kidney stones, Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray Analysis (EDX) to determine the mineralogy and the various chemical components in the kidney stones. The results show that the kidney stones are composed of five mineral groups and these are; mixed stone group, calcium oxalate, phosphate stone, uric acid, and cholesten. The cholesten has been diagnosed for the first time in Iraq, which might be linked to the diet type that includes high levels of cholesterol. Furthermore, the most common mineral group in the study area is the mixed stone which occurs in males more than females for the ages of 40 to 50 years old.

1. Introduction

Urinary stone disease, widely known as kidney stone (or renal stone), is a worldwide problem effecting nearly 3-5 % of the population which is mostly related to metabolic issues that can cause kidney function deterioration and it is a pathological bio-mineralization process in the urinary system [1,2,3,4,5,6].Urinary stones formation in kidney, bladder and urinary tract, is a complex phenomenon that is not clearly understood. Several factors contribute in the process of the kidney stone formation which could include metabolism disorder, infections, hormonal effects, dietary habits and reduce the fluids intake that increase the urine concentration and decrease its amount [7].Or could be related to genetics, lifestyle factors, drugs and some medical conditions that cause urine changes [8] or gender, climate, region geography [9,10,11,12,13,14]. Urine stones are formed when the concentration of certain substances increases, especially oxalate, uric acid, cysteine, calcium or deficiency of citrate in the urine [11,12, 14] and not eliminated from kidney and remain in the form of solid blocks in the kidneys or urinary tract. These crystals or lumps vary according to their size from small to medium and large, some of which are soft and fragile or solid [15,16]. Studying kidney stones crystallization processes is particularly important in order to understand the influence of the environmental factors on the metabolism process that causes their formation [17, 18]. Recent studies focus to explain the formation of urinary stones (urolithiasis) in the human body by identifying the causes of their spread [19]. The main mineralogical content of kidney stone is calcium oxalate group (i.e. whewellite and weddellite) and calcium phosphate with its end member's hydroxyapatite and urates [20] or other inorganic minerals such as struvite, brushite and rarely whilockite [21].

Several local studies were conducted to study the mineralogical composition of the kidney stones and their distribution [22, 23, 24, 25, 26]. The Calcium oxalate group represents the major mineral group identified [22, 23]. Urate stones, on the other hand, represent the main mineral contents in children patients [24]. Certain rare occurrences of dittmarite mineral and cysteine mineral were recorded in Iraq [23, 24, respectively]. These local studies shows that the main factors controlling the mineralogical variation of the studied kidney stone are related to geographical differences which reflect climatological influence along others such as genetic, physiological and pathological factors [25] or kidney stone mineralogy and patient's diet type [26]. This paper aims to examine the kidney stones derived from urolithiasis patients in Fallujah hospital, Fallujah city-western Iraq, to provide insights on their mineralogical composition. This research is part of the unpublished master thesis of the third author. Preliminary results are published by [26] and cited here for referencing purposes.

2. Methodology

A total of 20 samples (Figure 1) were collected - in 2017 from July to September from urolithiasis patients in Fallujah hospital. The patients were eleven males and nine females ranging in age between 30 and 62 years old. Each sample was processed with distilled water diluted hydrogen peroxide for 24 hours. Then sample preparation were conducted following [27] method. An infrared analysis was performed in the chemistry labs of the chemistry department, Faculty of Science / University of Kirkuk using an infrared spectroscopy device. The sample powder (0.5-2.0 mg) was taken with 100 mg of dry potassium bromide powder (KBr) then placed in the small pestle of the device and crushed the components. The sample was pressed into a 13mm transparent pellet by the pressure machine and analysed by the infrared spectrophotometer [28]. The test was conducted at a spectral range (4000-400 cm-1). The reflected rays from each granule with a specific wavelength appear in the form of peaks and are then interpreted using special tables designed for this purpose. Some samples were sent to University of Wollongong in Australia to validate the results.

The x-ray diffraction analysis of the studied samples was carried out at University of Wollongong-Australia using Philips Bragg - Brentano diffractometer (Philips PW 3710). The x-ray diffraction used the following analytical conditions: (Cu K) tube and (28.5mA) current and the voltage (35KV) and the wavelength (0.2Ao) and speed (1 mm / min). 2gm of the sample powder and placed in a minerals cell of the device which rotates at a different angle (from 2-70 degree). Moreover, the scanning electron microscope with EDX were used to identify the mineralogical components of the studied kidney stones in the University of Wollongong-Australia using the Phenom XL Desktop. Fresh fracture surface of the samples were coated by thin layer (¬ 10nm) of a conductive material, (i.e. gold) for SEM-EDX analysis using sputter coating. EDX analyses were run on representative spots to clarify their chemical composition to aid the mineral identification.

3. Results and Discussion

3.1- Mineralogy

3.1.1 X-ray diffraction (XRD) results

Table 1 shows the mineral composition of 20 samples from Fallujah city. The X-ray diffraction (XRD) results (Figure 2) show that the mineralogical composition of the studied kidney stones can be divided into five groups as follows:

•Calcium oxalate group which includes whewellite and weddellite minerals.

•Phosphate stone group which represented by struvite, newberyite and whitlockite.

•Uric acid mineral group represented by uricite mineral.

•Cholesten mineral group.

•Mixed combination of uricite/whewellite /whitlokite/weddellite minerals.

Table 1: Mineral contents of the studied kidney stones, data from XRD, After [26].

Major phase	Minor phase	Gender	Age(Year)	Sample No
Whewellite	N/A	Male Female	57 37	F1,F7
	Weddellite	Female	42	F11
	Uricite	Male	45	F15
	Uricite and Whitlockit	Male	39	F17
Uricite	N/A	Female	38	F4
	Whewellite	Male	52,42, 43, 62, 44	F3,F5,F12,F13,F14,F16
	Weddellite	Male Female	61, 40, 46 50,33	F8,F9,F18,F20
Cholesten	N/A	Female	37, 44	F10,F19
Struvite	Newberyite	Male Female	55 35	F2,F6

Table (2) presents the occurrence of each allocated mineral within the whole sampling of the studied kidney stones, which are classified into five groups. The two most common groups are mixed stone, consisting of uricite/whewellite/whitlokite/weddellite minerals (60%) and the calcium oxalate group, with a percentage of (15%).

Table 2: The occurrence of each allocated mineral within the whole sampling of the studied kidney stones After [26]

No.	Minerals group	Mineral member	The occurrences %
1	Mixed Stone	Uricite and Whewellite	40
		Uricite and Weddellite	15
		Uricite/Whewellite/Whitlokite	5
2	Calcium Oxalate	Whewellite/Weddellite	15
3	Cholesten Calculi	Cholesten	10
4	Phosphate Calculi	Struvite/Newberyite	10
5	Uric Acid	Uricite	5

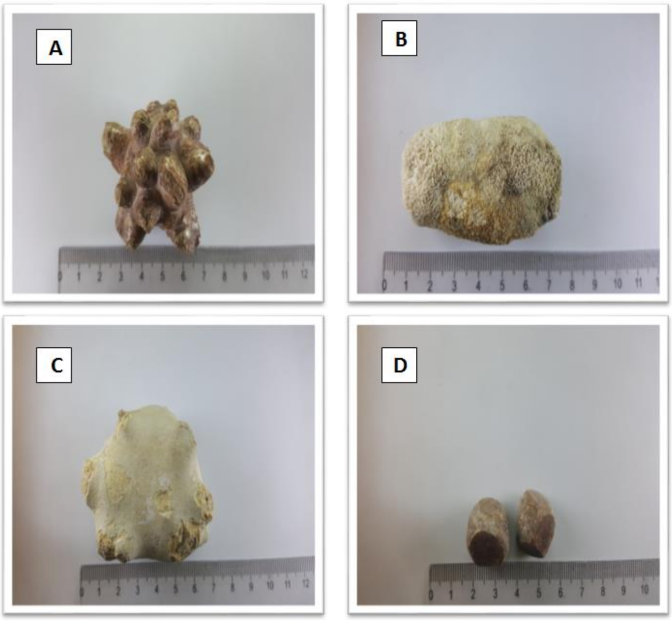

Fig. 1: Physical appearance of the studied surgically removed urinary stones, A: Calcium oxalate, sample F1. B: Phosphate stone, sample F2. C: Mix kidney stone composed from uric acid and calcium oxalate, sample F5. D: Cholestne, sample F10.

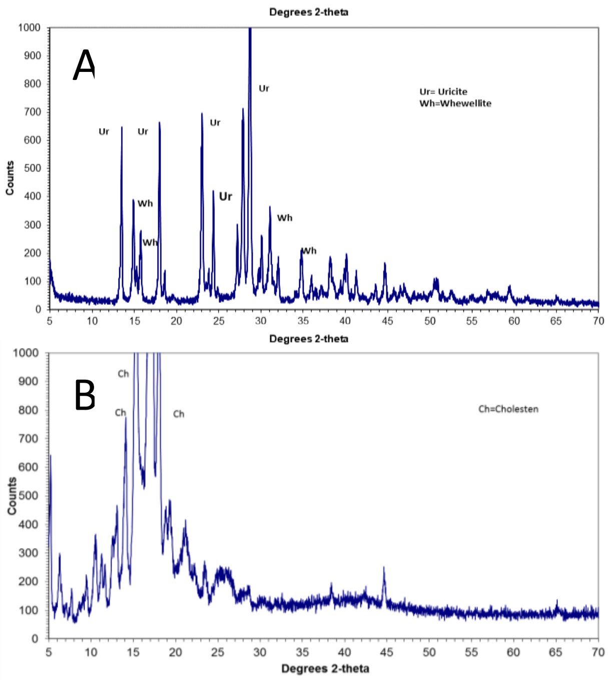

Fig. 2: Representative XRD patterns of the studied urinary stones, A: Uricite and Whewellite and B: Cholesten minerals.

3.1.2 Infrared spectroscopy (IR) analysis results

Table 3 and Figure 3 present the results of the infrared spectroscopy (IR) analysis, which shows five mineral groups:

1. Calcium Oxalate group of whewellite and weddellite minerals.
2. Uric acid group includes uricite mineral.
3. Cholesten group.
4. Phosphate stone group which includes struvite mineral.
5. Mixed combination of uricite and weddellite minerals.

The results of the infrared spectroscopy analysis show that mix stone composition of uric acid and calcium oxalate represent the main mineral composition in the studied samples (i.e. ten samples) with 50% while the other identified mineral constituents varied in percentage (see Table 3 for detail results).

Table 3: IR spectra results of the studied urinary stones along with minerals contents.

Samples	Spectra	Compound	Mineral	The occurrence %
F1,F7	3439 s*,3335 w*, 3030 w* 1698 s*,3483 s*,1100 w*	$CaC_2O_4.H_2O$	Whewellite	10
F2,F6	2936-3400 w*, 2916w 1005 sSh*,1652 w* 767 w*	$MgNH_4PO_4.6H_2O$	Struvite	10
F3,F5,F12,F13,F14 F15, F16,F17,F18,F19	3100 Sh*,3400 w*, 3005w*,700w*,3053s*, 1676s*	$C_5H_4N_4O_3$ $CaC_2O_4.H_2O$	Uricite Whewellite	50
F4	2770 w*,1732 s*,1345 Sh* 1308sSh*,1122 Sh*	$C_5H_4N_4O_3$	Uricite	5
F8,F9	2817 w*,1438-1348Sh* 1587s* * 3010w*,1671 Sh	$C_5H_4N_4O_3$ $CaC_2O_4.2H_2O$	Uricite Weddellite	10
F11	3510-3490s*, 3100 w*,1316sSh*	$CaC_2O_4.H_2O$ $CaC_2O_4.2H_2O$	Whewellite Weddellite	5
F10,F19	2930Sh*,1671 s*,1463s*,1022s*	$C_{27}H_{44}O_2$ or $C_{27}O_{76}O$	Cholesten	10
Total				**100**

*s =strong, *Sh=Sharp, *m= medium, *w=weak

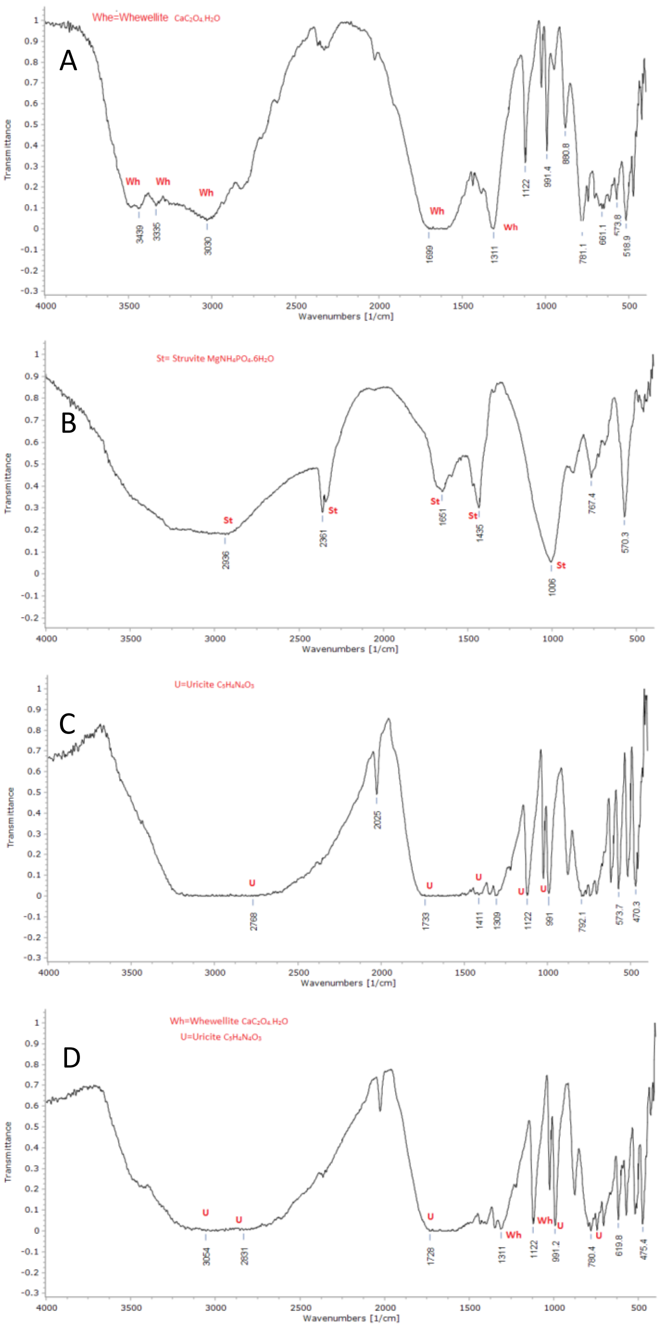

Fig. 3: Representative IR spectra of the studied urinary stones: A: Calcium Oxalate, B: Phosphate stone, C: Uricite mineral and D: mixed stone mineral group (Uricite and Whewellite).

3.1.3 Scanning electron microscope (SEM) results

Two types of phosphate stone were found, including magnesium ammonium phosphate (Struvite), whose crystals were randomly spaced, resembling wood panels formed by orthorhombic system, as in Figure 4. The second type of phosphate is the magnesium hydrogen phosphate group and its end member newberyite which has been observed in the form of semi-spherical granules as its granular forms are aggregated and have a tissue resembling a honeycomb like-shape as shown in Figure 4. Uric acid, which crystallizes in a monoclinic system, both uric acid crystals are prismatic and cluster like-shapes.

3.2 Mineralogical groups distribution by age and gender.

The prevalence of urinary stone recorded higher among urolithiasis patients aged 40-50 years old. The most common occurrence mineral assemblage in the studied area, is the mixed stone group (i.e. 60%, Table 2) which is more common in male patients than females. This differs from published work from other parts of Iraq which shows that calcium oxalate is the main mineral content of the studied kidney stones [22,23]. This could be related to geographical and climatological factors plus other factors such as diet types. The second most common mineral group is the calcium oxalate group which is represented by whewellite and weddellite minerals registered mainly in female patients. This could be related to the diet type which is high in calcium and oxalates such as legumes, salty foods and dairy products [23]. On the other hand, uric acid mineral group was identified with patients aged (30-40 years old) and predominately in females. This could be linked to high protein intake (e.g. red meat, legumes and eggs) [29]. Phosphate minerals stones were registered to be 10 % of the studied samples with no gender preference between two age groups (i.e. 30-40 and 50 -60 years old). Phosphate stones formation appears to be linked to blood acidity [30]. Patients' blood acidic control, which is between 60-6.5 and less than 7.0, helps prevent phosphate stone formation. Finally, cholestine group registered in females only ranged in age from 50 to 30 years old and could be linked to the high cholesterol level because cholestane mineral is basically composed from fat and lipids [31,32]. According to, samples can be categorized into four categories (Table 4) depending on the urolithiasis patient's age as follows;

- Category one: age ranges from 30-40 years old which includes six samples.
- Category two: age ranges from 40-50 years old and includes eight samples.
- Category three: age ranges from 50-60 years old and they are four samples.
- Category four: age ranges 60-63 years old with only two samples.

Male predominance (i.e. 65% of the total studied urolithiasis patients) is recorded within each age range except for those under 40 were female patients are more common. This could be due to increase rate of urinary tract infection in this age of sexually active group [33,34,35]. Meanwhile, the main reason of the increased male's infection with kidney stone is not clearly understood, although few researches are suggesting diet type. Males are more in favour of high protein and salty diet with lack of fluids and gaining weight which can leads to kidney stone formation [13].

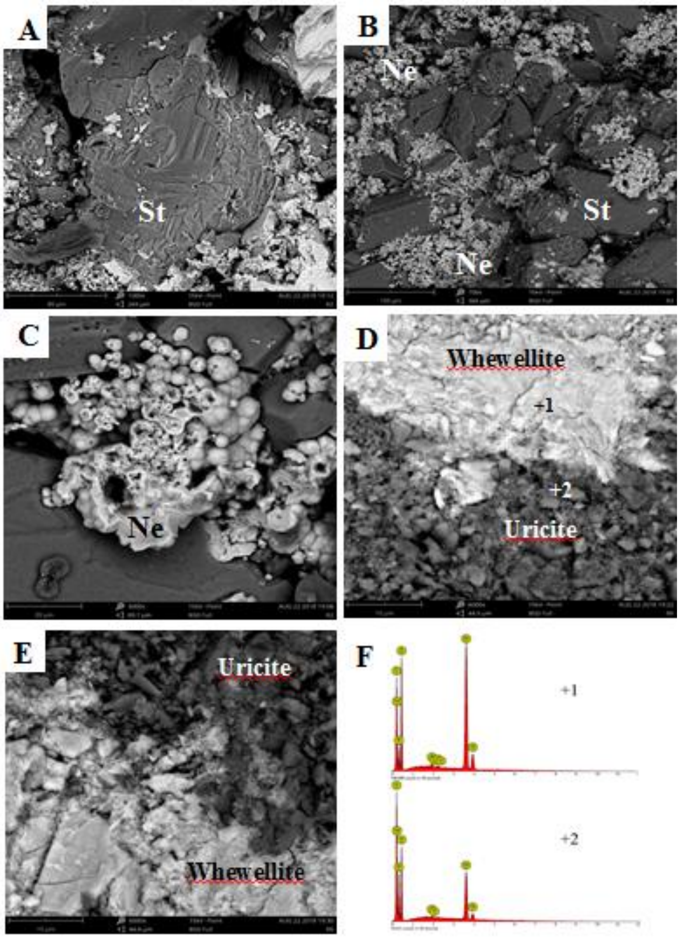

Fig. 4: Representative scanning electron micrographs (SEM) showing micro-structure of the studied urinary stones. A-B: Magnesium ammonium phosphate (woody-plate like shape of struvite mineral) and Magnesium ammonium phosphate (colloidal grains of newberyite mineral), sample F2. C: Magnesium ammonium phosphate (Newberyite) showing cluster of semi - honeycomb shape of newberyite mineral, sample F2. D-E: Prismatic crystals of uricite mineral and platy like shape of whewellite mineral, sample F5. F: EDX results of sample F5, analysis spots location are shown in image D above. *St=Struvite, *Ne=Newberyite, + = EDX analysis spot.

Male exposure to the kidney stone are more frequent due to the type of work the male conduct usually physical work which lead to more water exertion and sweeting and less urination in comparison to female which more water exertion take place from urination [36].Male in turn are subjected to stress and anxiety more than female which leads to increase in Vasopressin hormone that leads to decrease urine and crystal deposits and accumulate and kidney stone formation [37].

Table 4: The mineral groups distribution against urolithiasis patients age and gender.

Age Group	Calcium Oxalate		Uric Acid		Mixed Stone		Cholesten		Phosphate	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
30-40	1	-	1	-	1	1	1	-	1	-
40-50	1	-	-	-	1	5	1	-	-	-
50-60	-	1	-	-	1	1	-	-	-	1
60-62	-	-	-	-	-	2	-	-	-	-

4. Conclusions

The study identified five mineral groups of kidney stones: mixed stone group, calcium oxalate, phosphate stone, uric acid, and cholesten. It was also shown that the most common registered mineral group is the mixed stone group (i.e. 60%) which consists of uricite /whewellite /whitlokite/weddellite minerals. In addition, cholesten mineral was identified for the first time in Iraq within Fallujah city and this may be due to high-fat diet intake. The results of this study revealed that male predominance is recorded within each age range except for those under 40 were female patients are more common, this probably related to frequent urinary tract infection. In this study, diet is probably, among other factors like age and gender, the main controlling factor in forming kidney stones.

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دور المجهر الالكتروني الماسح وتقنيات التحليل الطيفي بالأشعة تحت الحمراء في تحديد معدنية حصى الكلى: مثال من مدينة الفلوجة ، غرب العراق

حسن احمد علي الجميلي ، عمر عادل محمد ، انوار سويد جاسم المعاضيدي

قسم الجيولوجيا التطبيقية , كلية العلوم , جامعة كركوك ، كركوك ، العراق

الملخص

تهتم الدراسة الحالية بمعدنية حصى الكلى للمرضى الراقدين في مستشفيات مدينة الفلوجة بمختلف الأعمار والأجناس. استخدمت تقنية حيود الأشعة السينية لمعرفة المكونات المعدنية لحصى الكلى، وتقنية الأشعة تحت الحمراء لمعرفة نوع المركبات الكيميائية الموجودة في كل عينة من حصى الكلى، و المجهر الالكتروني الماسح بتقنية تحليل مطيافية تشتت الطاقة بالأشعة لتشخيص المكونات المعدنية الرئيسية في حصى الكلى، أظهرت الدراسة وجود خمس مجموعات معدنية لحصى الكلى وهي مجموعة المعادن المختلطة، معادن أوكسالات الكالسيوم، حامض اليوريك، ومعادن الفوسفات فضلا عن مجموعة معادن الكولستين. تم تشخيص معدن الكولستين لأول مرة في العراق وهذا ربما يعود الى نوعية الغذاء الحاوي على الدهون بنسبة عالية. وان مجموعة الحصاة المختلطة شكلت أكثر المجاميع المعدنية إنتشارا في منطقة الدراسة والتي تصيب الذكور أكثر من الإناث وللأعمار من (40-50 سنة).