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Research Article

ELEMENTAL DISTRIBUTION ANALYSIS OF KIDNEY STONES

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Abstract

Various crystals are seen in human urine. Pathological crystals indicate crystal formation initiating urinary stones. Many crystals are not clearly identifiable under the ordinary light microscopy. The objective of the present study was to perform ICP, XRD and Scanning electron microscopic assessment of various urinary deposits and confirm the identity of elemental analysis of stones.

Keywords: Kidney stones, ICP, XRD, SEM.

Introduction

The most significant disease entity which comes under the category of biocrystallization is the human urinary calculi [1]. Renal calculi formation is one of the oldest diseases leading to hospitalization and surgery. In developed countries one in one hundred people has the problem of urolithiasis. It is estimated that about 4 to 15% of the male population in various countries and about half that many females will develop kidney stones. This makes it a social and economic problem of considerable magnitude. Variations in urine flow and tube blockage can cause local fluctuations in pH and concentration, increasing the probability of crystal deposition. Thus urine gets supersaturated and crystals nucleate, grow and aggregate resulting in the formation of calculi.

Calcium phosphates are the major constituents of bone and teeth. Dental calculus, brushitis, arthritis, etc., are the pathological conditions involving the deposition of calcium phosphates like minerals. Calcium phosphate may mineralize in different phases such as hydroxyapatite; tricalcium phosphate (whitelocklite), octacalcium phosphate and dicalcium phosphate dihydrate (brushite) in the order of increasing solubility. The investigation on the growth of calcium phosphates like brushite, monetite, Octa calcium phosphate, tricalcium phosphate and calcium hydroxyl apatite have received a considerable attention in connection with the formation of metabolic and non metabolic stones. Irusan et al, Sivakumar et al have grown these crystals using silica gel.

Crystallization of cystine results in cystinuria and cystinosis. Cystinuria is characterized by the excretion of large quantities of amino acids in urine. Cystinosis results in cystine stone and even in kidney damage. Cystine crystals can also occur in liver, spleen, thyroid glands, bone marrow and in ocular tissues. A hexagonal cystine crystals crystallized in laboratory has been reported by Girija et al [2].

Stone formation in renal systems is one of the oldest and the man common form of crystal deposition [3]. Analytical results show calcium oxalate (CaC_2O_4) to be

one of the major inorganic components of renal stones and is found to be present in almost all kidney and bladder stones [4]. About 39.5% of the total composition of the calculi is found to contain purely calcium oxalate and also it occurs along with calcium phosphates and apatite's [5]. It is well known that there are three different hydrated forms of calcium oxalate such as Calcium Oxalate Monohydrate (COM), Calcium Oxalate Dihydrate (COD) and Calcium Oxalate Trihydrate (COT). COD and COT are difficult to form urinary stone because they are unstable and easy to eject from human or animal body along with urine.

Among these Calcium Oxalate Monohydrate is found to be thermodynamically stable [6]. Though a number of researchers are tackling the problem from different angles, the mechanism of the formation of the stones is not yet clearly understood and a number of questions about the promoting and inhibiting factors still remain unanswered.

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 and calcium phosphate or a mixture of calcium oxalate and calcium phosphate [7]. Many stones are not homogeneous. Some have a nucleus of different composition from the surrounding.

Crystal formation can be caused due to several reasons [8]. 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 of crystals deposits can be evaluated using XRD, SEM, EDAX and ICP. The combined analysis of the above instruments formed the basis for the effective analysis of the stones grown by gel method.

Materials and Methods

The Forty six samples are collected from Stanley Medical College, Chennai and SP Hospital, Thanjavur. Most of the samples were very smaller in size, powdered in nature and ten samples which were in moderate size have been taken for characterization studies. The compositions of stones are determined by various analytical techniques such as Qualitative analysis, Elemental analysis (ICP) XRD and Scanning Electron Microscopy (SEM).

The concentration of cation and anion were analyzed by ICP analysis. The height and area of the peak were determined by these methods. The samples were analyzed and correlated with standard graph for the cations and anions.

The X-ray diffraction studies were carried out to determine the chemical constitution of stones. Powder XRD of the samples was carried out by PW1710 BASED diffractometer with CuKα radiation by using PC-APD diffraction software. From the diffraction peaks 2Ɵ values are correlated with hkl values and'd' space. The types of stones are identified.

Many of the relevant crystals are not recognized in ordinary microscopic analysis of urinary deposits performed in most of the clinical laboratories. They were gold sputtered to 100Aº and examined under SEM (Jeol JSM 35C microscope). 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. SEM can produce very high resolution images of a sample surface, about 1-5nm in size. The morphology, composition, etc., of crystal deposits can be evaluated using Scanning Electron Microscopy (SEM) together with elemental distribution analysis.

Results and Discussion

Characterization of stones by ICP

This ICP analysis is confirmed the confirmative and support evidence for US-I to US- X.

ICP – Analysis of Oxalate Predominate Stones:

In Cations, Standard Oxalate Graph is given Fig. 1. In Fig.1,the peak appeared at 32.13min represents the oxalate, the height and area of the peak is 9.91 mV and 709.755mV*Sec respectively which corresponds to the concentration of oxalate ion in solution is 10ppm.

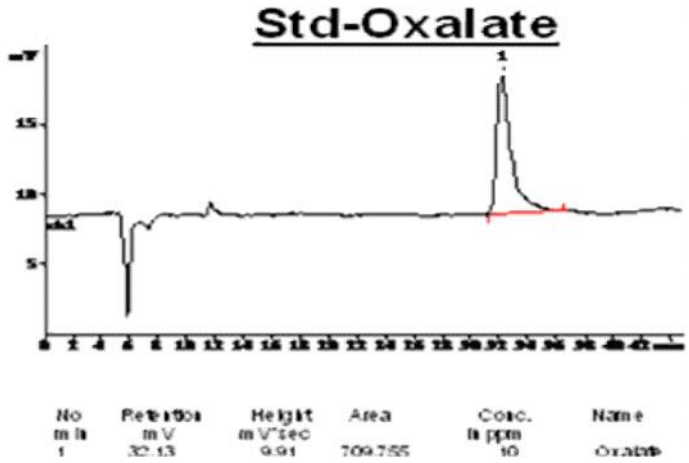

Figure.1. ICP for Std. Oxalate

With the help of the above standard graph, the samples of urinary stones are compared and the constituents of oxalate were determined.

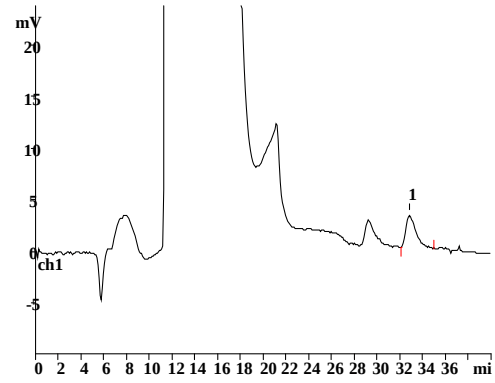

Figure. 2 ICP for US – I

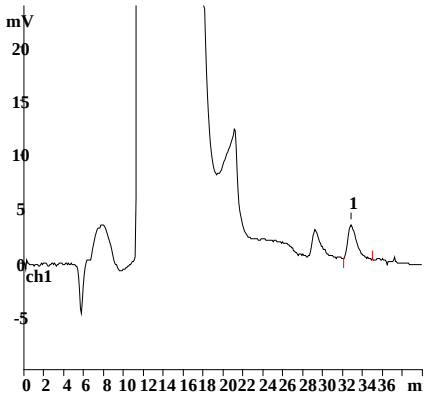

Figure. 3 ICP for US-III

ICP for the sample US-I and III is given in fig. 2 and 3. A characteristic peak has appeared at 32.87min and 32.13min with the height of 3.13mV and 9.10mV, area 197.158mV*sec and 716mV*Sec for the concentration of 278ppm and 10ppm respectively. When ICP for US-I and III were compared with standard ICP for oxalate it was found that the characteristic peak for oxalate were found to be same.

Table: 1 ICP Data for Oxalate Stone

Name of the sample	Retention mV	Height Mv	Area mV*Sec	Concentration ppm (Oxalate)
US-I	32.87	3.13	197.15	278
US-III	32.13	9.10	716.22	10
US-IV	33.01	3.03	197.05	278
US-X	32.80	3.23	197.25	278

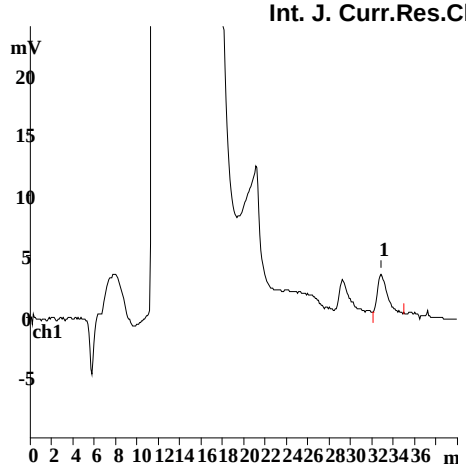

Figure. 4 ICP for US – IV

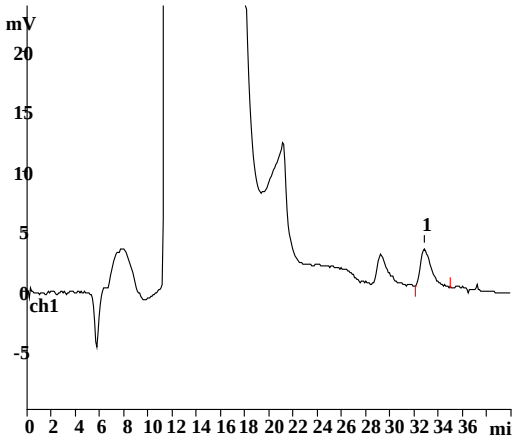

Figure.5 ICP for US – X

In Fig. 4 and 5, the ICP for Samples US – IV and X, at concentration 278ppm, has a peak at 33.01min and 32.80min with the height of 3.03mV and 3.23mV and area of 197.058mV*sec ,197.25mV*sec respectively. On comparing the standard ICP for oxalate with ICP for US-IV and US-X, it was found that the major constitution was oxalate.

ICP – Analysis of Phosphate Predominant Stones

ICP analysis of the stones confirms the constitution of the cations and anions. In Cations, Phosphate Graph is given by Figure. 6.

STD - PHOSPHATE

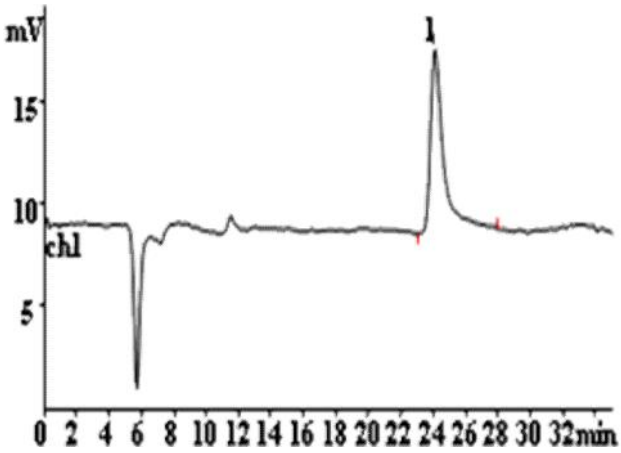

Figure.6 ICP for Std. Phosphate

In Fig.6, the retention time of the peak for the standard Phosphate is 24.04mV, the height and the area of the peak are 9.01 mV ,517.155mV*sec respectively, for concentration of 10ppm.

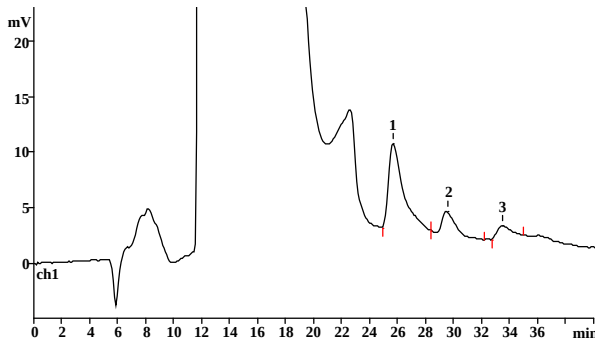

Figure: 7 ICP for US-II

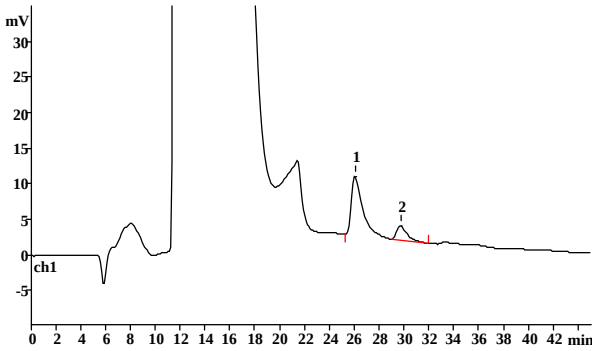

Figure. 8 ICP for US – V

In Fig. 7 the ICP for sample US –II, at concentration 1075ppm and 100ppm, have peaks at 25.71min and 33.52mV for phosphate and oxalate with the height of 7.68mV and 1.13mV and the area of the peaks are 556.172mV*sec and 70.749mV*sec respectively. On comparing the Standard Oxalate and Phosphate with ICP for US-II it was found that phosphate and oxalate was confirmed.

Table: 2 ICP data for Phosphate Predominant Stones

Name of the sample	Retention mV	Height mV	Area mV*Sec	Concentration ppm
US-II	25.71	7.68	556.17	1075 (Phos.)
	33.52	1.13	70.74	100(Oxalate)
US-V	26.05	8.24	519.4	1083(Phos.)
	29.69	1.98	115.79	9.2 (Oxalate)
US-VI	26.91	4.43	295.47	1118 (Phos.)
	30.68	2.26	148.70	9.5(Oxalate)
US-VII	27.07	10.1	582	1126 (Phos.)
US-VIII	27.07	13.97	15.52	1126 (Phos.)
US-IX	27.07	15.52	990.7	1126 (Phos.)

ICP for the sample US-V and VI are given in Fig. 8 and 9. The characteristic peaks has appeared at 26.05min,29.69min and 26.91min,30.68min with the height of 8.24mV,1.98mV and 4.43mV,2.26mV and area 519.440mV*sec,115.792mV*sec and 295.47mV*Sec, 148.70mV*Sec for the concentration of 1083ppm and 1118ppm respectively. When ICP for US-V and US-VI was compared with standard ICP for Phosphate and Oxalate it was found that the characterisation peak for Phosphate and Oxalate were found to be same as that of the standard.

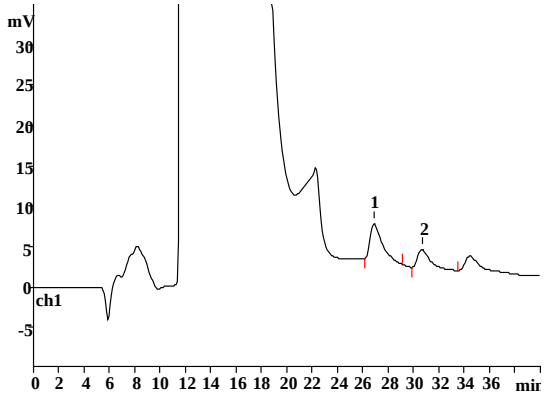

Figure. 9 ICP for US – VI

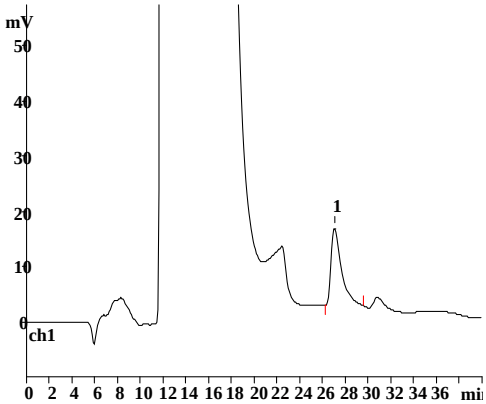

Figure. 10 ICP for US – VII

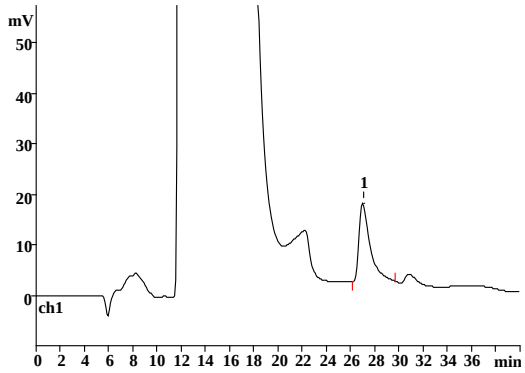

Figure. 11 ICP for US – VIII ‘

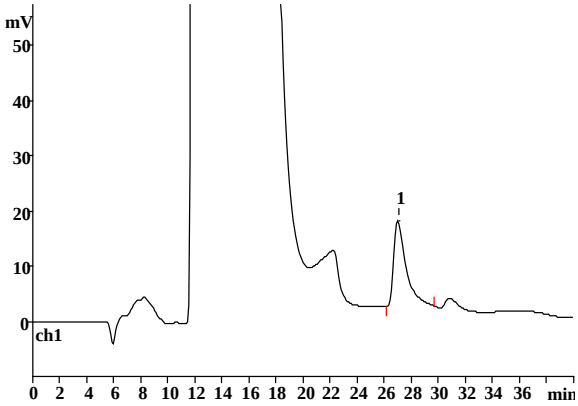

Figure. 12 ***ICP for US – IX***

Fig. 10 to 12 represents the ICP for sample US – VII,VIII and IX. A characteristic peak appeared at 27.07min with the height and area of the peak of 13.97 mV,15.52mV and 889.265mV*sec, 990.7mV*sec respectively for the concentration of 1126ppm for all the three samples. Phosphate is confirmed in all these samples.

Generally in a Oxalate stone found that the characteristic peak has appeared at 33min to 33min with the height and the area of 3mV to 10mv and 197mV*Sec to 716mV*Sec and for the concentration of 278ppm to 10ppm. Phosphate stone found that concentration 1075ppm to 1126ppm, has peak 25.71min to 27.02min with the height of 4.43 mV to 15.52mV and area of 295mV*Sec to 556mV*Sec.

Characterisation stones by X-Ray Diffraction Studies (XRD)

The Powder X-ray diffraction studies are analyzed the purity and identification of the compound can be determined by indexing hkl values with the standard materials, standard ASTM values of the substance. Hence it gives additional supportive evidences for the samples.

XRD – Analysis of Oxalate predominat Stones

XRD patterns of the oxalate samples for US-I, III, X and IV are in figure.13 to 16 are shown below.

Table: 3 XRD Data for Oxalate predominant stones

2Θ	D spacing	hkl value
14.26	5.94	200
20.01	4.49	211
24.15	3.65	002
32.17	2.78	411
37.21	2.42	103
40.00	2.21	213

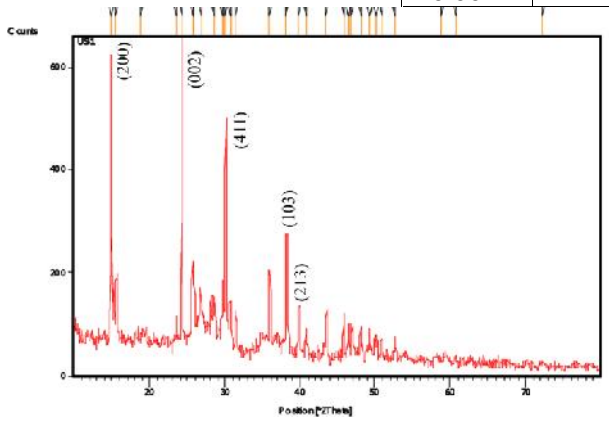

Figure: 13 XRD for US- I

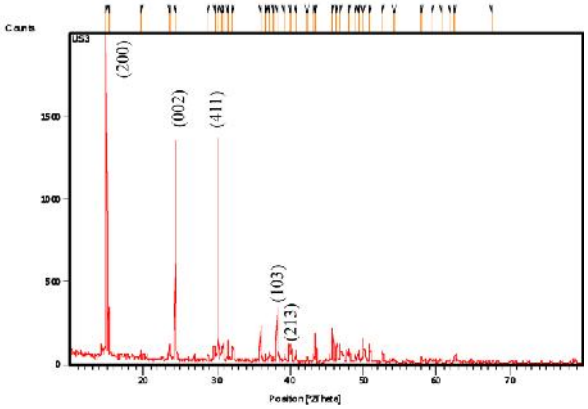

Figure: 14 XRD for US- III

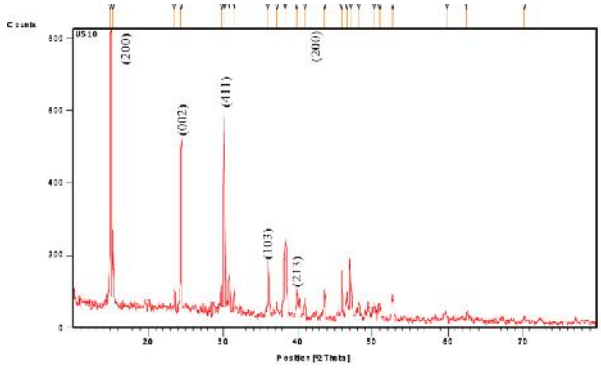

Figure. 15 XRD for US- X

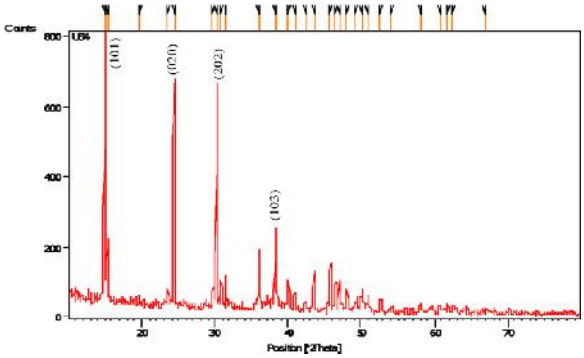

Figure. 16 XRD for US- IV

In Figure. 13 to 15 for US-I,III and X , crystal obtained the following diffraction peaks (2Ɵ):14.26,20.01,24.15,32.17,37.21 and 40.00 which can be correlated to the (hkl) indices (200),(211),(002),(411),(103) and (213) of Calcium oxalate dihydrate.

In Figure. 16 for US-IV, crystal obtained the following diffraction peaks(2Ɵ): 14.95,24.39,30.12 and 38.13 which can correlated to the (hkl) indices (101),(020),(202) and (130) of calcium oxalate monohydrate.

XRD –Analysis of Phosphate Predominant stones

An X-Ray diffraction study also confirms the constitution of the Phosphate stones. In figure.17 to 22 are US-II,V,VI,VII,VIII and IX . In Phosphate stones the 2Ɵ values are correlated to hkl values and d-spacing.

Table: 4 XRD Data for Phosphate predominant stones

2Ɵ		D spacing		hkl value
Std.Phos.	**Sample**	**Std.Phos.**	**Sample**	
10.8	10.5	8.16	8.36	100
16.8	16.5	5.26	5.39	101
21.7	21.5	4.08	4.14	200
22.8	23.0	3.88	3.85	111
25.8	25.8	3.44	3.43	002
28.9	29.0	3.08	3.07	210
31.7	31.9	2.81	2.80	211
32.9	33.3	2.71	2.68	300
32.9	33.7	2.71	2.65	202
35.4	35.8	2.52	2.50	301
42.3	42.4	2.13	2.12	302
46.7	46.3	1.94	1.95	222
50.5	50.7	1.80	1.79	321
54.4	54.6	1.68	1.67	104
57.1	57.8	1.61	1.59	313
69.7	69.9	1.34	1.34	512

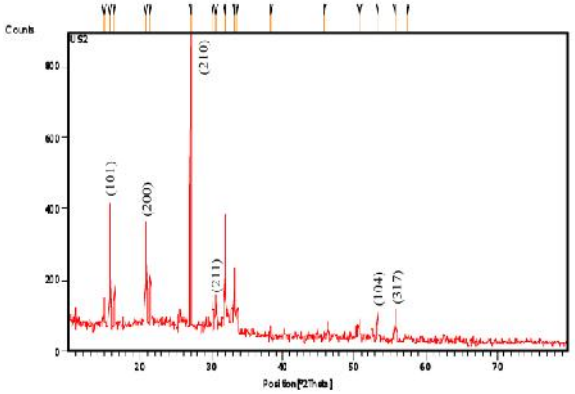

Figure:17 XRD for US- II

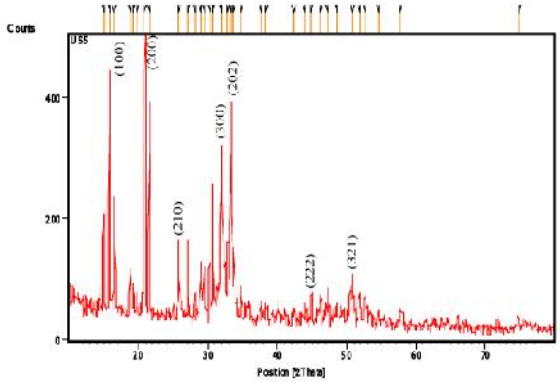

Figure:18 XRD for US- V

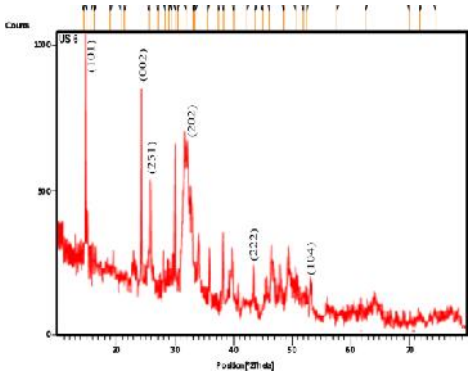

Figure:19 XRD for US- VI

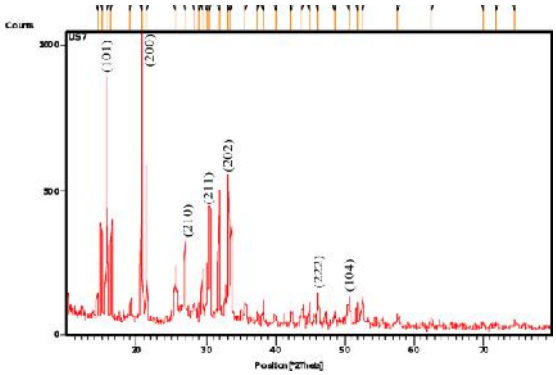

Figure:20 XRD for US- VII

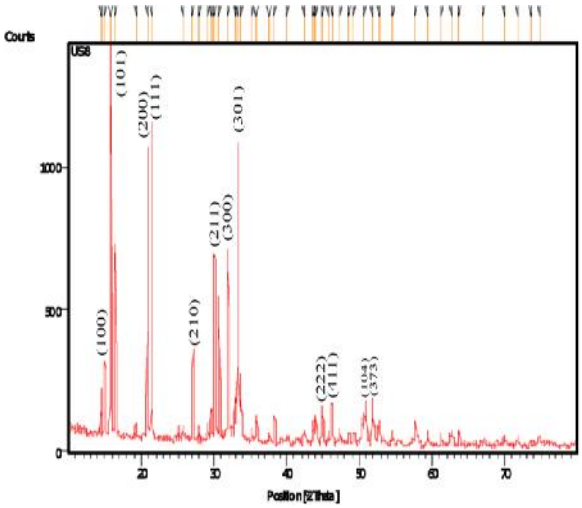

Figure:21 XRD for US- VIII

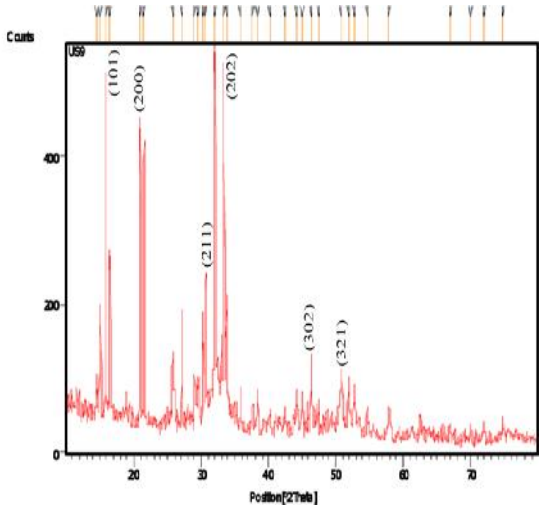

Figure:22 XRD for US- IX

The mixture of Phosphate and Oxalate crystals obtained displays the following diffraction peaks (2Ɵ): 10.5, 16.5, 21.5,23.0, 25.8, 29.0, 31.9, 33.3, 33.7, 35.8, 42.4, 46.3, 50.7, 54.6, 57.8 and 69.9 which can correlated to the (hkl) indices (100), (101),(200),(111), (002),(210),(211),(300) (202), (301),(302),(222), (321),(104),(313) and (512) of phosphate phase.

Powder XRD techniques confirmed the functional groups and crystalline phases of the Calcium Oxalate Monohydrate , Calcium Oxalate Dihydrate and Hydroxy apatite crystals in the Urinary stones.

Characterisation of stones by Scanning Electron Microscopy (SEM)

Present study was to perform Scanning Electron Microscopic assessment of various urinary stones. The morphology, composition, etc., of crystal deposits can be evaluated using microscopy and scanning electron microscopy (SEM) together with elemental distribution analysis. 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 electronics. Hence it is possible to identify the crystal, however small it may be, through SEM. SEM can produce very high resolution images of a sample surface, revealing details about 1-5 nm in size. The Urinary stones 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. They were gold sputtered to 100 Aº and examined under SEM (JEOL JSM 35 C microscope). In-depth analysis of urinary stone was possible using SEM as electrons were allowed to fall on the sample. When urinary stones were seen, their morphology was recorded by taking photographs at different angles. SEM could give a maximum magnification of 50000 times. Photographs were taken at different magnifications (30-5000) and were recorded.

The use of optical microscopy and SEM combined permits a more far-reaching investigation in modern research areas. The description of some findings, with the aid of examples, explains the efficiency of the system in the investigation of urinary calculus analysis.

SEM – Analysis of Oxalate Predominant Stones

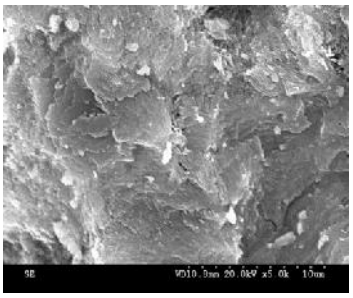

Figure.23 SEM for US- I

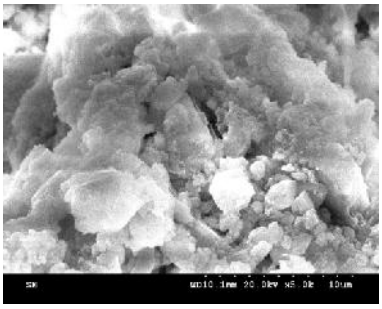

Figure.24 SEM for US- IIIF

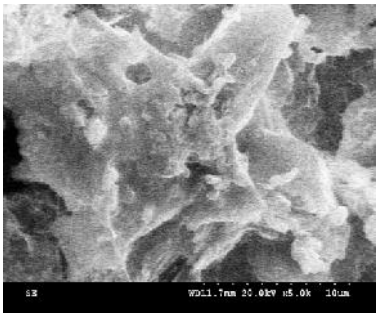

Figure.25 SEM for US- IV

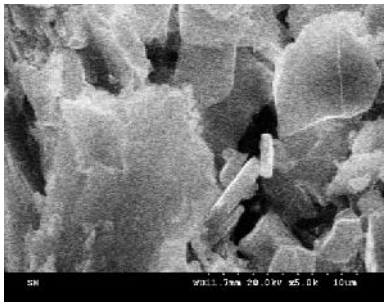

Figure.26 SEM for US- IV

Fig. 23, 24 and 26 represents the morphological analysis using SEM of the samples of urinary stones US-I, III and X respectively which are spherical and plate shape. This result shows the presence of calcium oxalate monohydrate stones. Fig.25 represents the morphological analysis using SEM of the sample of US – IV, which has rectangular pyramidal shape. This result shows the presence of calcium oxalate dihydrate stone.

SEM – Analysis of Phosphate Predominant Stone

Fig. 27 and 29 represents the morphological analysis using SEM of the samples of Urinary stones US- II and VI respectively which are plate like star shaped crystals. This result shows the presence of Phosphate dihydrate stone

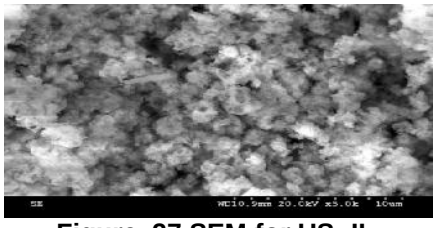

Figure. 27 SEM for US- II

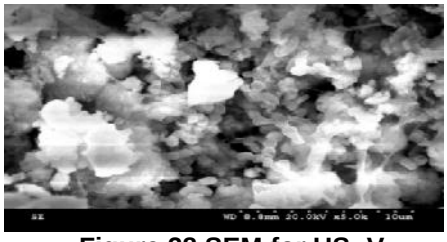

Figure.28 SEM for US- V

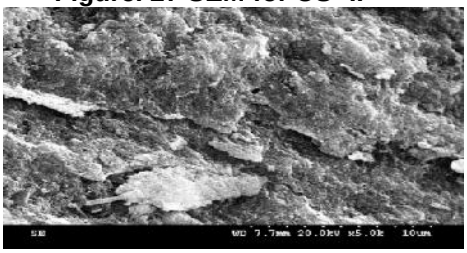

Figure. 29 SEM for US- VI

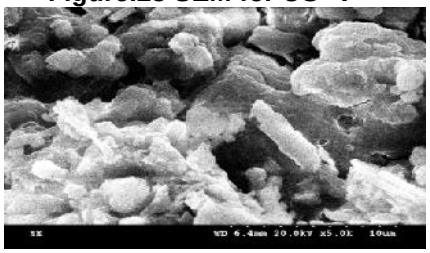

Figure. 30 SEM for US- VII

Fig.28 and 30 represents the morphological analysis using SEM of the samples of US – V and VII which

are spherical and star shaped crystal. This result shows the presence of oxalate monohydrate and Phosphate dihydrate stone.

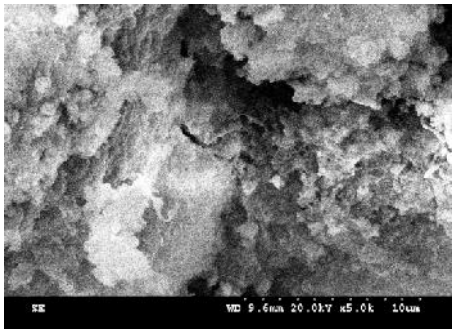

Figure. 31 SEM for US- VIII

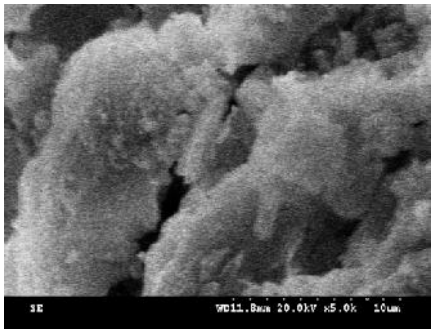

Figure. 32 SEM for US- IX

Fig. 30 and 31 represents the morphological analysis using SEM of the samples of Urinary stones US- VIII and IX respectively which are plate like star shaped crystals. This result shows the presence of Phosphate dihydrate stone.

Morphological studies of all the urinary stones by SEM, reveals that the Oxalate Monohydrate, Oxalate Dihydrate and Phosphate stones are having spherical shape, rectangular plate and star shaped crystals respectively. On comparison with the shape, the corresponding constituents of urinary stones of the samples US – I to US-X were depicted. The correlation from the morphology also supports that US-I,III,IV and X stones are oxalate predominant with little phosphate concentration and US-II,V,VI,VII,VIII and IX stones are phosphate predominant with less oxalate concentration.

Conclusion

The Characterizations of urinary stones of the samples US-I to US-X were carried out by ICP,XRD,SEM andEDAX studies and the results the following conclusions were revised.

ICP analysis also confirms oxalate and phosphate concentration in the urinary stones.

Powder XRD techniques confirmed the functional groups and crystalline phases of the Calcium Oxalate Monohydrate , Calcium Oxalate Dihydrate and Hydroxy apatite crystals in the Urinary stones.

Morphological studies of all the urinary stones by SEM, reveals that the Oxalate Monohydrate, Oxalate Dihydrate and Phosphate stones are having spherical shape, rectangular plate and star shaped crystals respectively. On comparison with the shape, the corresponding constituents of urinary stones of the samples US – I to US-X were depicted. The correlation from the morphology also supports that US-I,III,IV and X stones are oxalate predominant with little phosphate concentration and US-II,V,VI,VII,VIII and IX stones are phosphate predominant with less oxalate concentration.

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