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College of Science

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Chemical Analysis of Kidney Stone

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الآية

قال تعالى:

(وإذا مرضت فهو يشفين)

صدق الله العظيم

سورة الشعراء الآية (80).

Dedication

To our parents, who inspired us with faith in Allah, love and respect towards all people who sincerely helped us in work.

To our families, to our friends and our colleagues with our love

Acknowledgements

Thanks to Allah,

First, we would like to express our thanks to our supervisor D.Mohamed El Mukhtar Abdel Aziz for his3 interest in the research project and guidance and help during the research.

Our thanks are extended to the staff of Ibn Sina and Sharge Alneel Hospital and to the technicians of University of Khartoum laboratories.

Abstract

The purpose of this study its verification from computation and types of kidney stones, carry out from Sudanese patients so collected 6 samples (4 males 2 females) and analysis the samples with many spectroscopy, x-Ray flronunces (XRF), Infra-red spectroscopy (IR) in the area range (4000-500nm) and ultra violet spectroscopy (UV) and statistical analysis of data of patients of kidney stones from (2010-2015) from ShargeAlneel hospital by spss21 .

The analysis of element by (XRF) to determine concentration of elements Ca, Cu, Zn, Sr, Ni, Pb, Br, Fe, and found that the concentrations high in Ca, Zn and Fe

Result of (IR) indicates that 80% of the samples consist of calcium oxalate. IR show that the wave length for all samples in the range (220-2230nm).referring statistical analysis that males more commonly than females to from stones and the age that have the ability to from the stones in the range 18 years and aboveand the presence of the stones in the kidney more than its in the ureterand bladder and Khartoum state more areas spread of the kidney stones.

المستخلص:

الغرض من هذه الدراسة هي التحقق من مكونات وانواع حصاوي الكلي المستخرجة من المرضي السودانيين حيث تم جمع 6 عينات (4 ذكور، 2 اناث) وتم تحليل العينات بعده مطيافات، مطيافيه الأشعةالسينية(XRF) والأشعة تحت الحمراء (IR) في المنطقةالواقعة بين (4000_ 500) nm ومطيافية الاشعة فوق البنفسجية (UV) واجراء التحليل الاحصائي لبيانات مرضى حصاوى الكلى للسنوات من (2010 _ 2015) من مستشفى شرق النيل باستخدام التحليل الاحصائي (SPSS 21) واجراء التحليل للعناصر بجهاز XRF لتحديد تراكيز العناصر كالسيوم، النحاس، الخارصين،الاسترانشيوم، النيكل، الرصاص، البروم والحديد . ووجد ان التراكيز عالية في كل من الكالسيوم، الزنك والحديد.

نتيجةIR تشير الي ان 80 % من عينات الحصاوى تكون من اكسلات الكالسيوم.UV تدل علي ان الطول لكل العينات في المدى 220-230nm

واشاره التحليل الإحصائي ان الذكور اكثر شيوعا من الاناث لتكوين الحصاوى والاعمار التي لها القدرة علي تكوين الحصاوى في المدى 18-70 سنه و وجود الحصوة في الكلية اكثر مما هو في الحالب والمثانة وان ولاية الخرطوم اكثر مناطق انتشر فيها الحصاوى .

Contents:

Subject	Page No.
الآيـة	
Dedication	
Acknowledgements	
Abstract	
المستخلص	
Contents	
List of Tables	
List of Figures	
Chapter One	
1- Introduction	1
1-1Urinary System	1
1-2 Kidney Stone	2
1-2-1 Causes of Kidney Stone	2
1-2-2 Types of Kidney Stone	2
1-2-3 Kidney Stone Symptoms	3
1-2-4 Treatment of the Kidney Stones	3
1-2-5 Prevention kidney stones	4
1-3Problem	4
1-4 previous studies	6
1-4-1 Hiba Hassan Taha Ali Mikial (2012) Analysis of kidney stones in Ibn Sina Hospital	6
1-4-2 NaseemAslamChanna ' Analysis of kidney stone by FTIR spectroscopy	7
1-5 Objectives	8

Chapter Two Experimental section	
2– Materials and Methods	9
2-1 Materials	9
2-1-1 Samples Collection	9
2-1-2 Chemicals	9
2-1-3 Instrument	10
2-1-3-1Infrared spectrophotometer	10
2-1-3-2Ultra Violates spectrometer	10
2-1-3-3 X-ray florescence	10
2-2 Methods	10
2 -2 -1 IR Experiment	10
2 -2 -2 UV Experiment	11
2 -2-3 XRF Experiment	11
2 -2 -4 Statistical Analyses	11
Chapter three Results and discussion	
3-1Elemental analysis by X-Ray fluorescence (XRF)	12
3-2 Elemental analysis by Infra red spectroscopy (IR)	19
3-3Ultra violet	29
3-4 Statistical analysis results	36
Conclusion	42
Recommendations	43
References:	44
Appendices	

List of Tables

Table No	Content	Page No.
Table 1	Sample collection	9
Table 2	Concentrations of different elements in stone samples	12
Table 3	IR elemental analysis of kidney stones	19
Table 4	Ultra violet spectra o fkidney stone samples	29
Table 5	Statistical analysis of kidney stones patients from ShargeAlneel hospital	36

List of Figures

Figure No	Content	Page No.
Figure 1	Urinary System	1
Figure 2	XRF of Sample 1	13
Figure 3	XRF of Sample 2	14
Figure 4	XRF of Sample 3	15
Figure 5	XRF of Sample 4	16
Figure 6	XRF of Sample 5	17
Figure 7	XRF of Sample 6	18
Figure 8	IR Standard of sturvite	19
Figure 9	IR Standard Uric Acid Stone	20
Figure 10	IR Standard of of calcium oxalate	21
Figure 11	IR standard of sycetine	22
Figure 12	IR Spectra of Sample 1	23
Figure 13	IR Spectra of Sample 2	24
Figure 14	IR Spectra of Sample 3	25
Figure 15	IR Spectra of Sample 4	26
Figure 16	IR Spectra of Sample 5	27
Figure 17	IR Spectra of Sample 6	28
Figure 18	UV Spectra Sample 1	30
Figure 19	UV Spectra Sample 2	31
Figure 20	UV Spectra Sample 3	32
Figure 21	UV Spectra Sample 4	33
Figure 22	UV Spectra Sample 5	34
Figure 23	UV Spectra Sample 6	35
Figure 24	gender of patients	37
Figure 25	Patients Resident	38
Figure 26	Ages of Patients	39
Figure 27	Place of stones	40

Chapter One

1- Introduction

1-1Urinary System

The urinary system is a group organs in the body concerned with filtering out excess fluid and other substances from the blood stream . The substances are filtered out from the body in the form of urine .The urine is a liquid produced by the kidney collected in the bladder and excreted through the urethra .

Urine is used to extract excess minerals or vitamins and blood corpuscles from the body . The urinary organs include ; the kidney, bladder, urethra and ureters. The female and male urinary systems are very similar , they differ only in the length of the urethra. (Graaff)

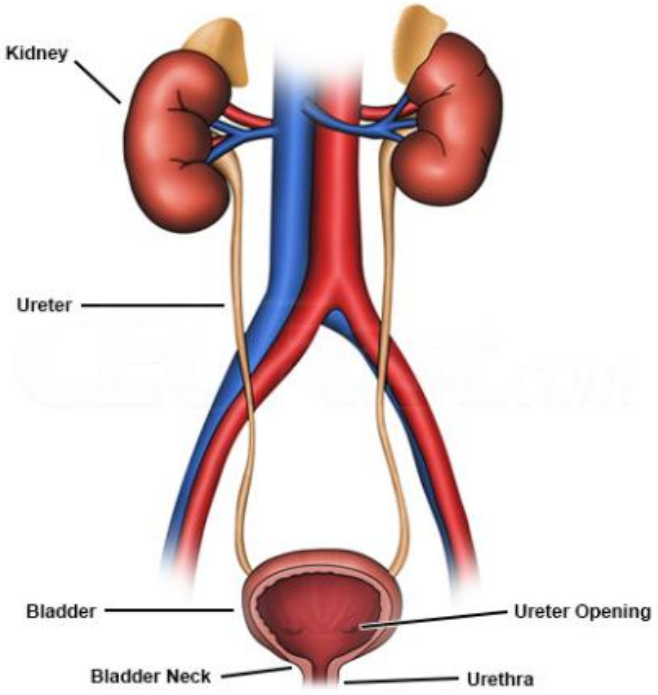


Fig 1**Urinary System**

1-2 Kidney Stone

A kidney stone is a solid piece of material that forms in a kidney when substances that are normally found in the urine become highly concentrated.

A stone may stay in kidney or travel down the urinary tract. Kidney stones vary in size. A small stone may pass on its own ,causing little or no pain. A larger stone may get stuck along the urinary tract and can block the flow of urine, causing severer pain or bleeding.(Saurin 2010)

1-2-1 Causes of Kidney Stone

Kidney stone can form when substances in the urine such as calcium oxalate and phosphors become highly concentrated. Certain foods may promote stone formation in people who are susceptible , but scientists do not believe that eating any specific food causing stone to form in people who are not susceptible will form stones . People who do not drink enough fluids may also be at higher risk as their urine is more concentrated.(Leye Ajayi 2007)

1-2-2 Types of Kidney Stone

Four major type of kidney can form:

1-Calcium Stone

Are the most common type of kidney and occur in two major form
Calcium oxalate calcium phosphate.
Calcium oxalate stones are more common. Calcium oxalate formation may be caused by high calcium and high oxalate exertion. Calcium phosphate are caused by the combination of high urine calcium and alkaline urine meaning that the urine has a high pH.(Raju 2008)

2-Uric Acid Stones

Form when the urine is persistently acidic. Addict richin purines substance found in animal protein such as meals، fish and shellfish may increase uric acid in urine. If uric acid becomes concentrated in the urine it can settle and form a stone by itself or a long with calcium.

3-Struvite Stone

Result from kidney infection eliminating infected stones from the urinary tract and staying infection free can prevent struvite stones .(Knight 2010)

4-Cystine Stone

Results from genetic disorder that causes cysteine to leak through the kidney and into the urine forming crystals that tend to accumulate into stone.(Raymond 2009)

1-2-3 Kidney Stone Symptoms

People with kidney stone may have pain while urinating , see blood in the urine or feel as sharp pain in the pack or lower abdomen. The pain may take short or long time. People may experience nausea and vomiting with the pain. However, people who have small stone that pass easily through the urinary tract may not have symptoms at all.(Leye Ajayi 2007)

1-2-4 Treatment of the Kidney Stones

Treatment of kidney stones usually depends on their size and what they are made of as well as whether they are causing pain or obstructing the urinary tract

Kidney stones may be treated by agonal practitioner or by an urologist doctor who specializes in the urinary tracts small stones usually pass through the urinary without treatment. Still the person may need pain medication and should drink lots of fluids to help move the stone along. Pain control may cons to fluids or intravenous medication, depending on the duration and severity of the pain.

The fluids may be needed if the person becomes dehydrate from vomiting or from an inability to drink

A person with a larger stone or one that blocks urine tow and causes great pain may need more urgent treatment such as :-

1-Shock wave lithotripsy

A machine called lithotripter used to crush the kidney stone

2-ureteroscopy

Aureteroscopy a long tube like instrument with an eye piece is used to find and retrieve the stone with small basket or to break the stone up with laser energy

1-2-5 Prevented kidney stones

The first step in preventing kidney stones is to understand what is causing the stone to form the health care provider may ask the person to try to catch the kidney stone as it passes so it can be sent to lab for analysis stones that are retrieved surgically can also be sent to lab for analysis

The health care provider may ask the person to collect urine for 24 hours after stone passed or been removed to measure daily urine volume andminrallerds.

Producing too little urine or having a mineral abnormally can make person more likely to form stone.

Kidney stone may be prevented throuh change in eating diet and nutrition and medication.

Eating diet and nutrition:

People can help prevent kidney stones by changing their fluid drink. Depending on the type of kidney stone a person has changes in the amount of sodium animal protein، calcium and oxalate consumed also help ، drinking enough fluids each day is the best way to help prevent most types of kidney stones

Health care recommends that a person drinks 2 to 3 liters of fluids a day. People with cystine stones may need to drink even more, though water is best other fluids may also help to prevent kidney stones, such as citrus drinks

Recommendations based on the specific type of kidney stone include the following:

Calcium Oxalate Stone:

Reducing sodium

Reducing animal protein such as meat

Getting enough calcium from food

Avoiding foods

Calcium phosphate stones:

Reducing sodium

Reducing animal protein such as meat

Getting enough calcium from food or taking calcium supplements with food.

Uric Acid stones:

Limiting animal protein

1-3Problem:

The kidney stones are common diseases in human, and injury ratio in men greater than women.

This study will be conducted to identify the components and types of kidney stones .Moreover, the ratio spread will be determined and admpts to prevent its development in the future

1-4 Previous studies

There are many studies in this area carried by:

1-4-1 Hiba Hassan Taha Ali Mikial (2012) Analysis of kidney stones in Ibn Sina Hospital

Ten samples were collect after surgery (3 females and 7 males, age range 2 to 70 years) kidney stone samples analyzed by several spectroscopic methods inductively coupled plasma, optical emission spectroscopy (ICP/ES), X-Ray fluorescence (XRF), Fourier transform Infra red spectroscope techniques (FTIR),in the region (500-4000nm) and ultraviolet spectroscopy (UV)

Statistical analysis was carried out for data of patients with urinary stones for the years from 2007 to 2011 from Ibn Sina hospital by using spss program

The analysis of elemental by ICP was carried out to determine the concentrations of this element Sodium (Na) potassium (K)Calcium (Ca) Magnesium (Mg), Manganese (Mn), Cupper (Cu), Iron (Fe), Zinc (Zn), Chromium (Cr) and Molybdenum (Mo), elements.

Elements Mg , Ca ,Na, and K were found at high concentration more than the other elements Elemental analysis by XRF was carried out to determine the concentrations of the element present in the same samples.

The results of IR spectra indicate that 80% of stone samples were composed from calcium oxalate and UV spectra show that the wave length of all samples appeared the special range 233-257 nm

Statistical analysis indicated that males are likely to form kidney stones than females and the age that have more ability to form stones in the range 16-60 years

1-4-2 NaseemAslamChanna ' Analysis of kidney stone by FTIR spectroscopy

Kidney stones recovered from patients of different hospitals of Hyderabad and adjoining areas (Liaquat University hospital Jamshoro, Memon Chaitable Hospital Hyderabad, Wali Bahai Rejputant Hospital Hyderabad Naseem Medical Center Hyderabad and Isra University hospital Hyderabad) (Sindh ,2006)

A total of 58 kidney stones samples were collected randomly and analyzed for composition by Fourier transform Infrared spectroscopy (FTIR) it was found that 37.9 % were pure calcium oxalate , 3.4 % were uric acid , 44% calcium oxalate + uric acid 3.4% calcium oxalate and 10.3% were magnesium ammonium phosphate . The IR bands were compared with standards . Gender wise comparison revealed that majority of the stones (68.9%) analyzed were recovered from male patients , whereas stones recovered from females were only 31.1% . Age wise comparison data disclosed that age range for the presentation of kidney stone disease was 15-29 years

1-5 Objectives

This study was carried out to determine the composition and identity the type of kidney stone in Sudanese population, in order to decrease the number of kidney diseases in the future.

Chapter Two

Experimental section

2– Materials and Methods

2-1 Materials

2-1-1 Sample Collection

The stone samples were surgically recovered from 6 (4 males and 2 females, having age range 20-60 years) patients visiting ShargAlneel hospital and Ibn Sina hospital

Data of patients:

Table 1 sample collection

Sample no	Sex	Age	Resident	Place of stones	Water	Main food
1	female	23	Alsalama	Kidney	wells	Dura
2	male	54	Omdurman	Bladder	Nile	Dura
3	female	28	Jabra	Kidney	wells	Milk
4	male	58	Giad	Bladder	Health water	Chicken
5	male	35	Zalengi	Kidney	wells	Dura
6	male	60	Shsrgalneel	Kidney	wells	Wheat

2-1-2 Chemicals:

Hydrochloric acid (36% - 38%)

Nitric acid (69% – 72%)

Anhydrous potassium bromide

Distilled water

2-1-3 Instrument

2-1-3-1Infrared spectrophotometer

Infrared spectroscopy is certainly one of the most important

analytical techniques available to today' for sscientists. One of the great advantages of infrared spectroscopy is that virtually any sample in virtually any state may be studied (Stuart ., 2004)

2-1-3-2Ultra Violates spectrometer

The UV spectra were obtained for dilute solution (1 mg in 100 ml of solvent) apportion of this solution was transferred to silica cell.

matched cell containing pure solvent was prepared and each cell placed in the appropriate place in the spectrometer this is so arranged that two equal beams of light are passed one through the solution of the sample one through the pure solvent the intensities of the transmitted light are then compared over the whole wavelength range of the instrument (Dudley1935)

2-1-3-3 X-ray florescence

Stone samples were applied as powder the concentration of the element was determined in ppm.

2-2 Methods

2 -2 -1 IR Experiment

For KBr pellet, a 0.2 g of dried potassium bromide was weighed and the sample (0.05 g) was added. the mixture was homogenized for 2 minutes , then pressed into disk and introduced to the instrument .

2 -2 -2 UV Experiment

The sample (0.5 g) was placed into aqua regia (6 ml conc. HCL and 2 ml conc. HNO3) was added, Water was added and diluted to 25 ml .1 ml of this solution was taken and more demonized water was added to complete volume to 100 ml . The spectra were then recorded.

2 -2-3 XRF Experiment

The sample (1.0 g) was placed into pellets using 15 pressing machine, the diameter of each pellet was about 2.5 cm , the pellets were introduced to the XRF spectrometer

2 -2 -4 Statistical Analyses

Data of stones were collected from Sharg Alneel Hospital during the period 2011 -2015; the data were analyzed by the SPSS 21 program.

Chapter three

Results and discussions

3-1Elemental analysis by X-Ray fluorescence (XRF)

(XRF) was carried out to determine the concentrations of the element in samples

Table 2concentrations of different elements in stone samples

sample	1 ppm	2 ppm	3 Ppm	4 ppm	5 ppm	6 Ppm
Ca	320000	8760	79400	318000	391000	313000
Cu	15.6	12.4	12.2	12.7	22.1	16.6
Zn	85.3	30.2	16.9	91.5	214	742
Sr	96.7		148	40.8	110	142
Ni		59.8	55.2			
Pb		10.3	10.6			
Br			9.68		24.6	
Fe					367	

Calcium was present in all samples at high concentration, Zinc was the second element in all samples, Iron came after Calcium and Zinc in sample 5 and others were not detected with XEF.
The elements Ca,Cu and Zn were found in all samples; Sr was found in all samples expect sample 2; Ni was found in samples 2,3 ; Pb was found in sample 2,3,6; Br was found in samples 3,5 and Fe was found only in sample 5.

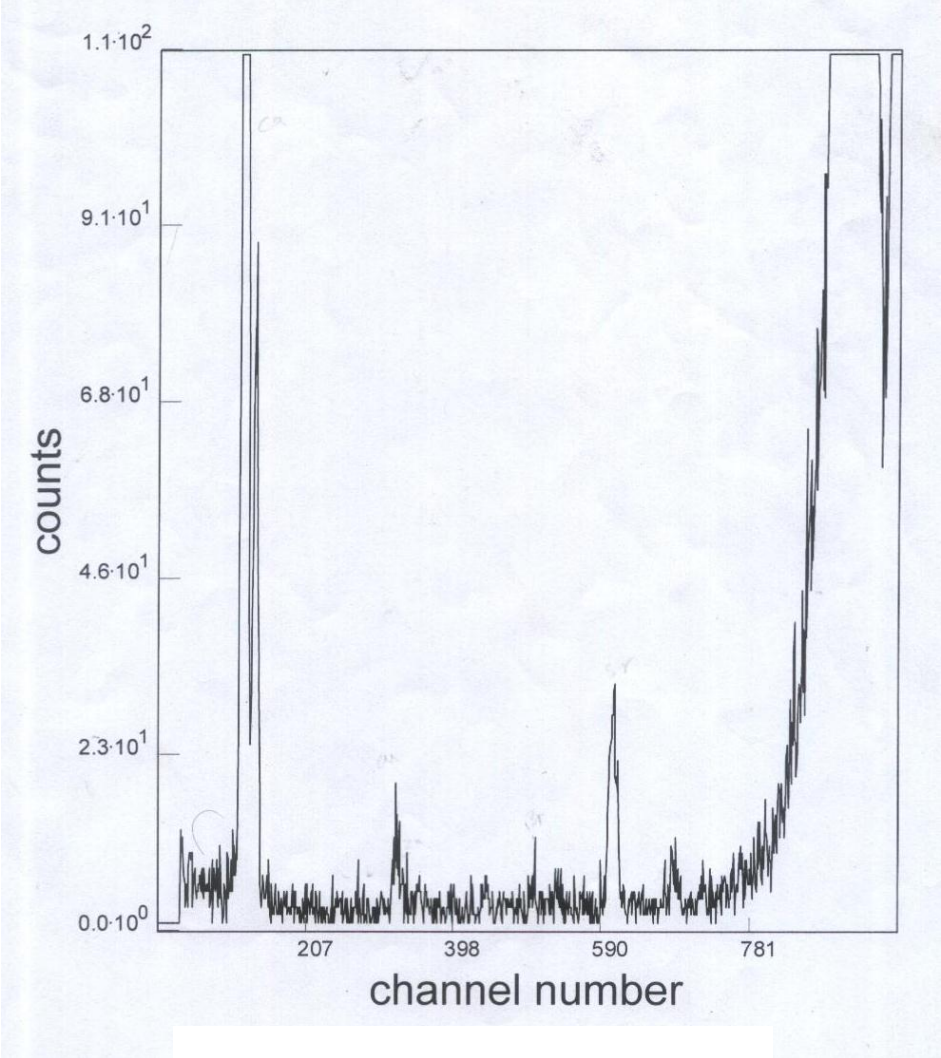


Fig 2 XRF of sample 1

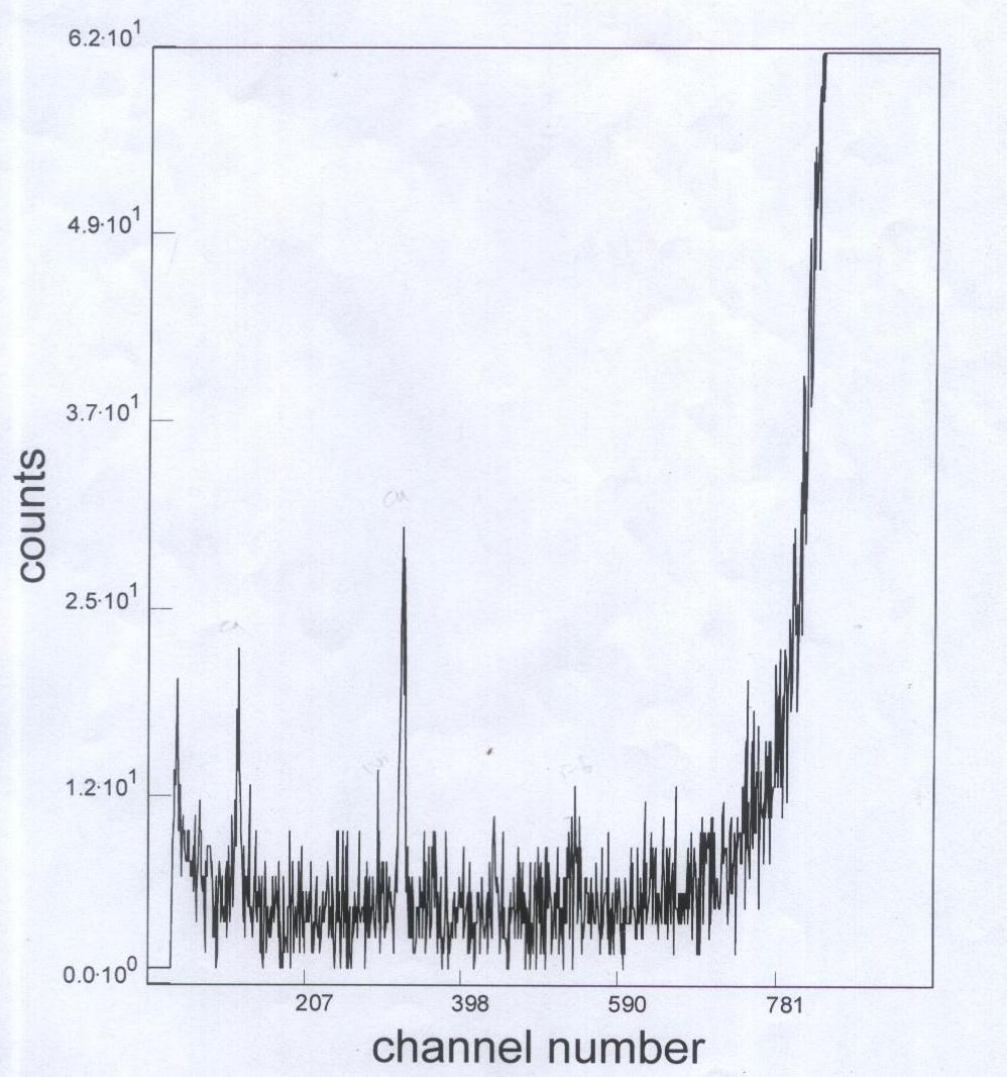


Fig 3 XRF of sample 2

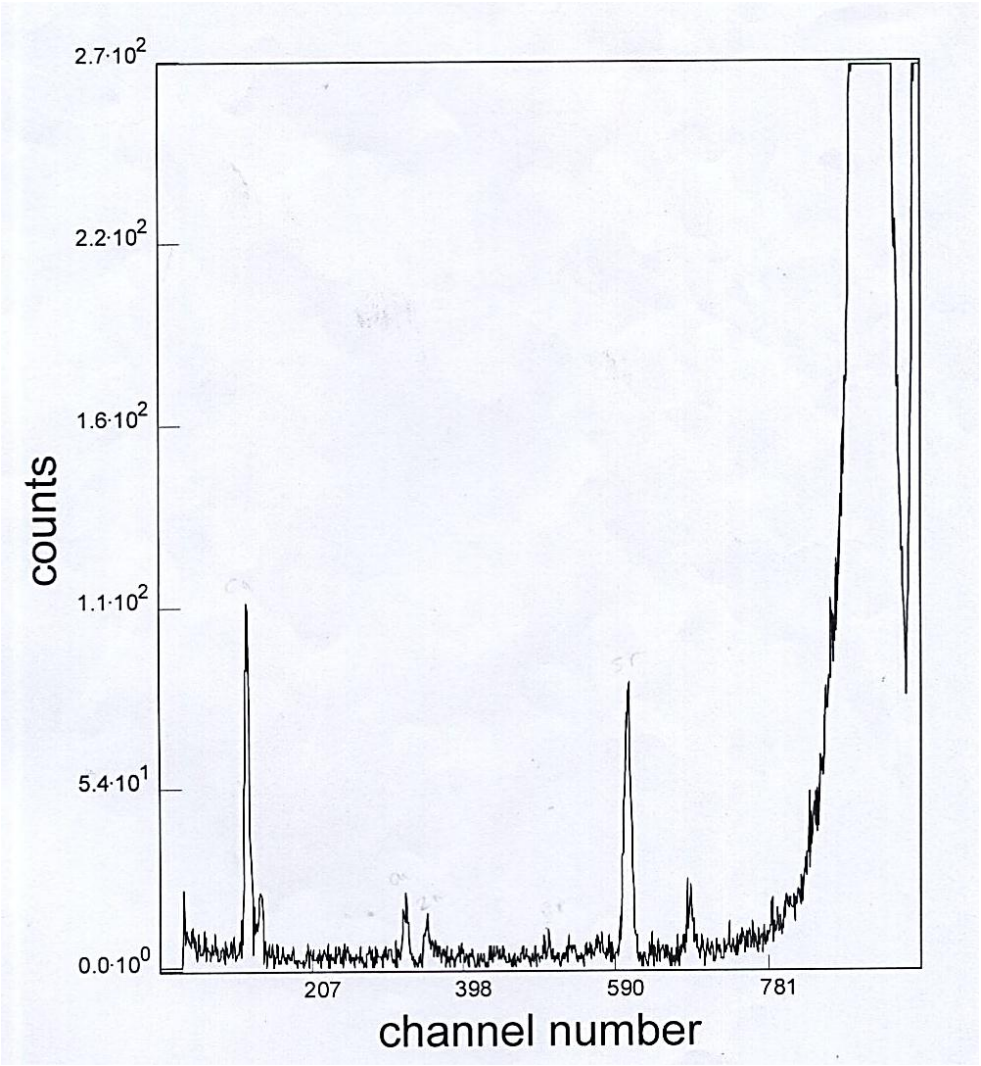


Fig 4 XRF of Sample 3

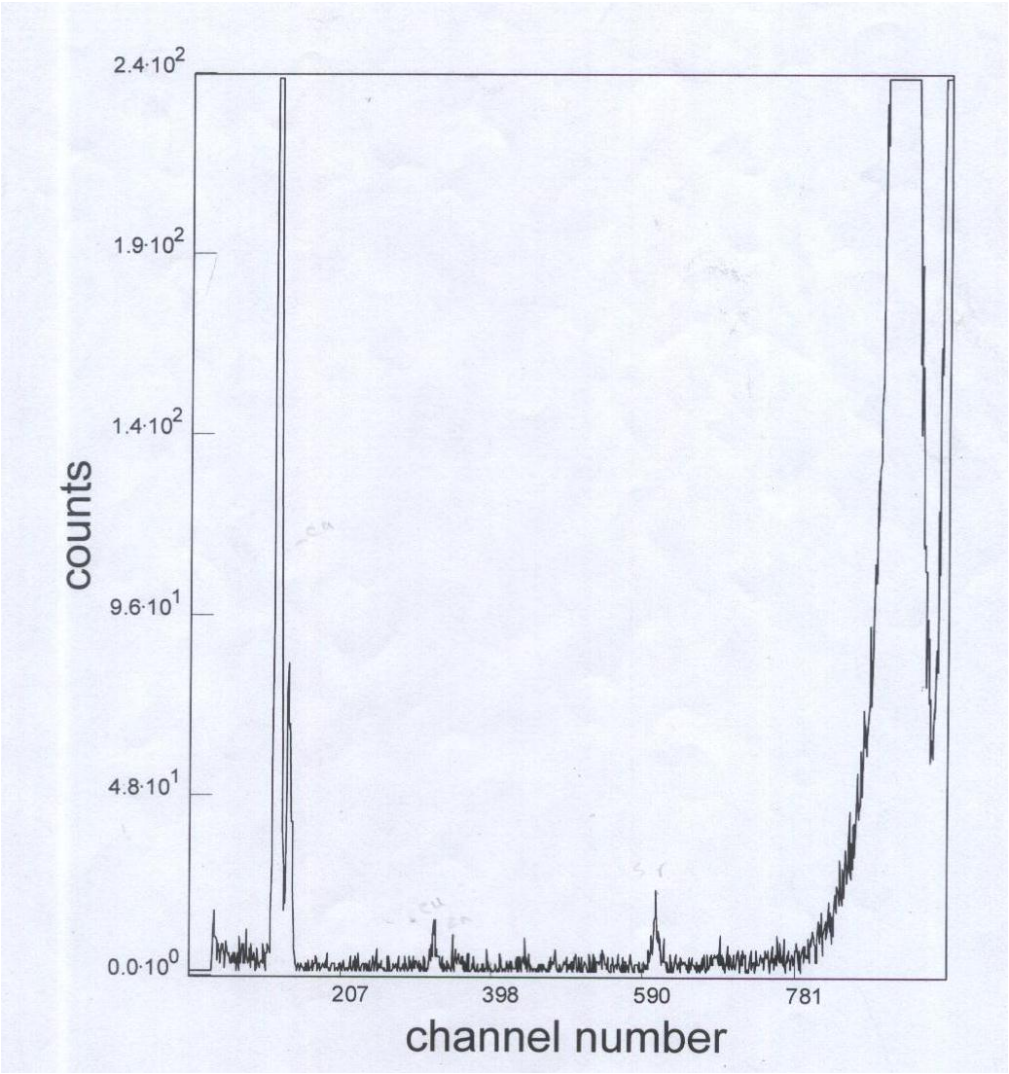


Fig 5 XRF of sample 4

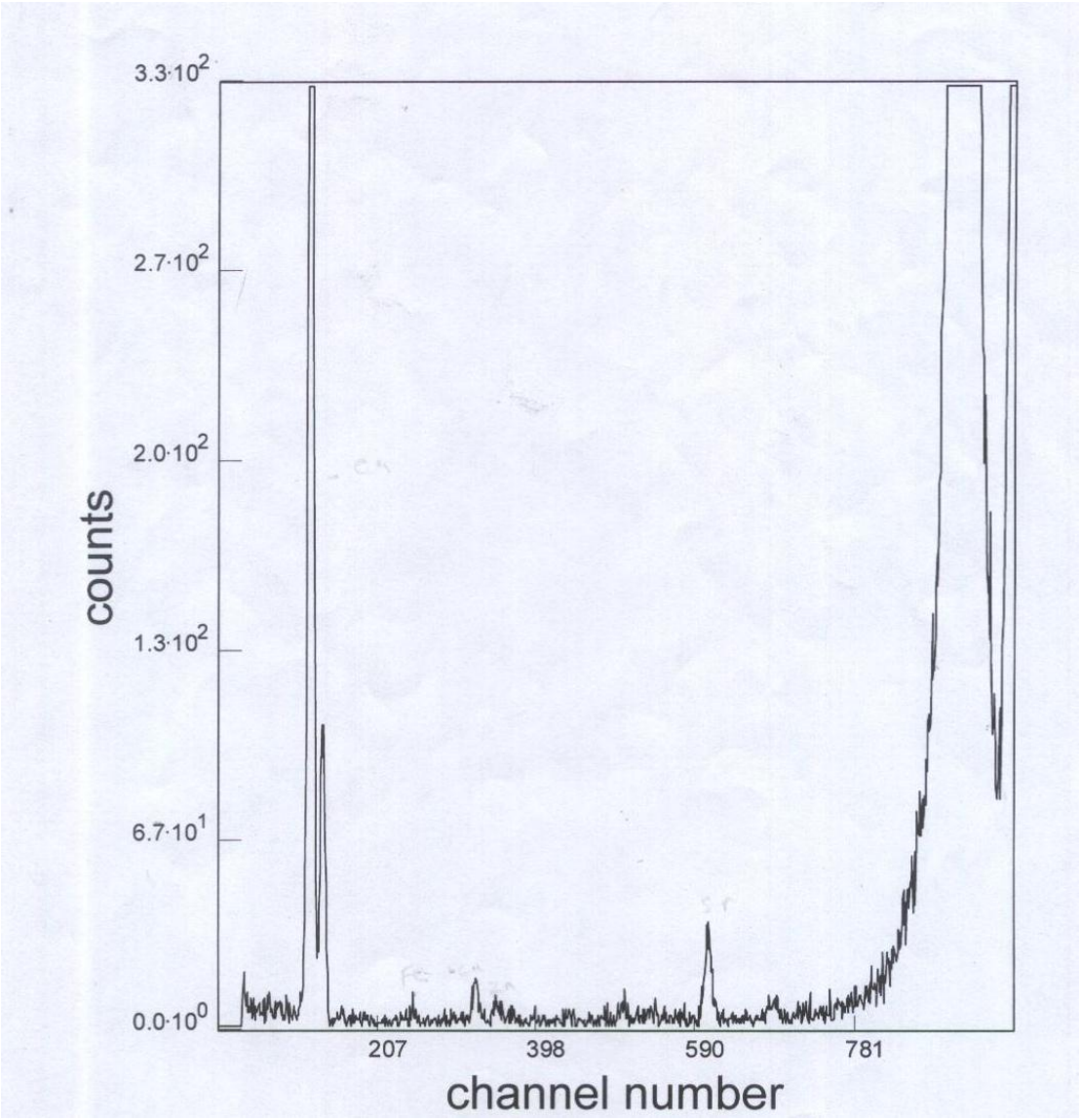


Fig 6 XRF of Sample 5

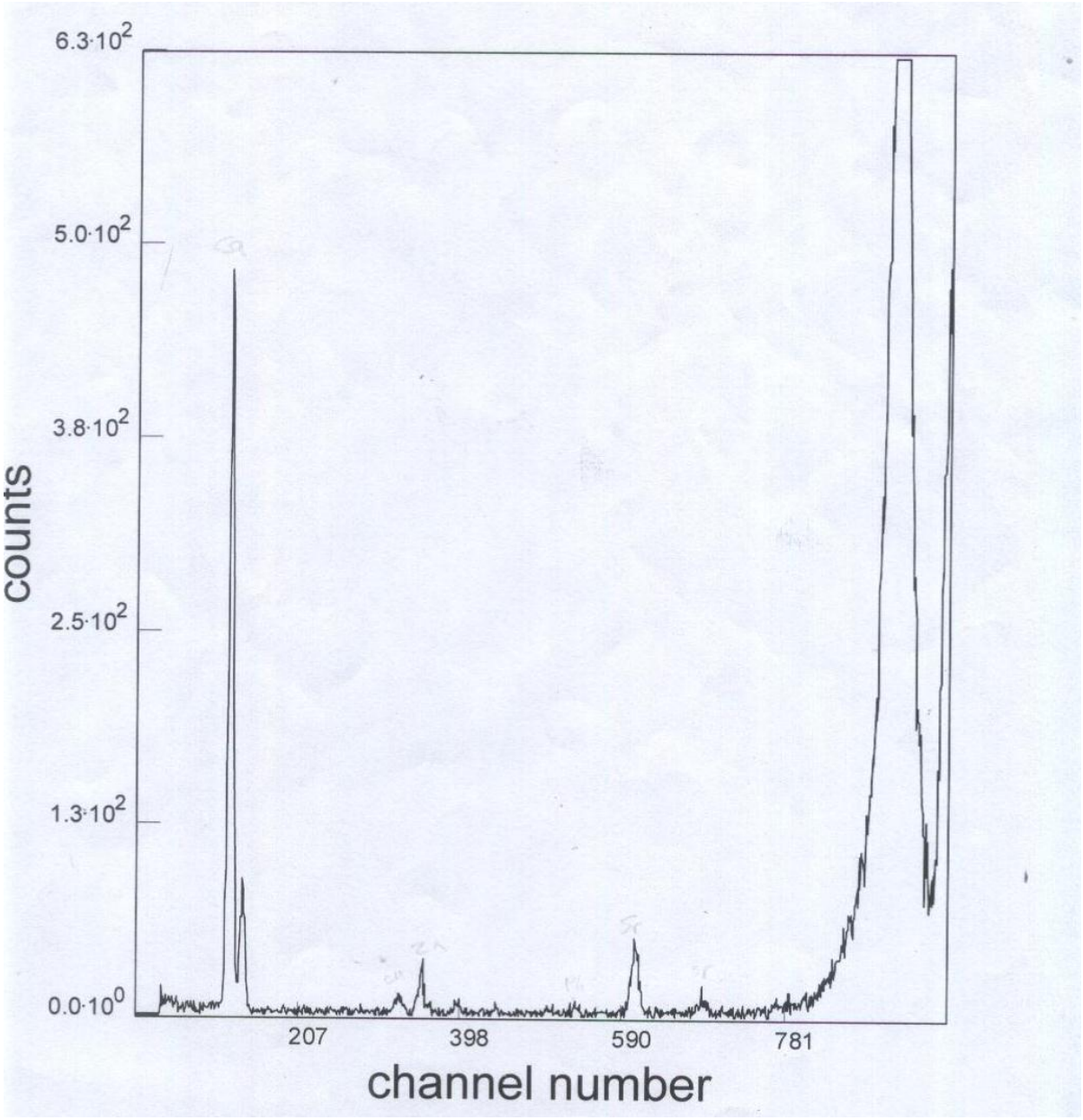


Fig 7 XRF of Sample 6

3-2 Elemental analysis by Infra red spectroscopy (IR)

IR was carried out to determine the types of kidney stones

Table 3 elemental analysis of identity stones

Functional Group / samples	C=o Asystr 778.53	C-C Symstr 1314.93	OC=O Aysstr 1604.64	C=C Str 1637.29	N-H ben 1018.13	C-N Straro 738.03	N-H str 3362.63	NH3 ben 1469.19	P-OC str 970.53
1	779.12	1318.23	1620.49				3434.5	1397.4	950.53
2	784.48	1304.67	1675.21				3013.7	1402.83	989.87
3				1638.5	1011.67	770.23			
4	779.42	1317.62	1619.35				3331.09		948.91
5	779.27	1317.77	1619.43				3338.45		950.49
6	779.7	1319.77	1628.8						

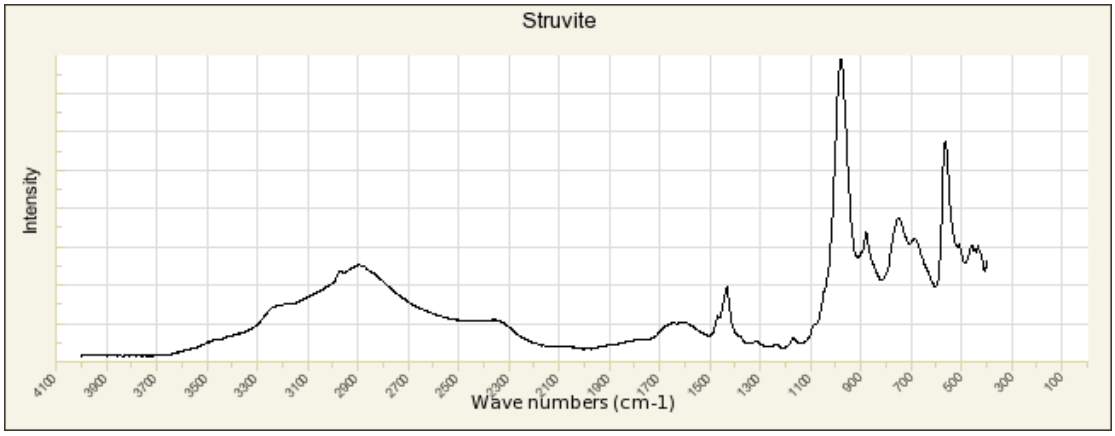


Fig 8 Typical FTIR spectrum of sturvite standard

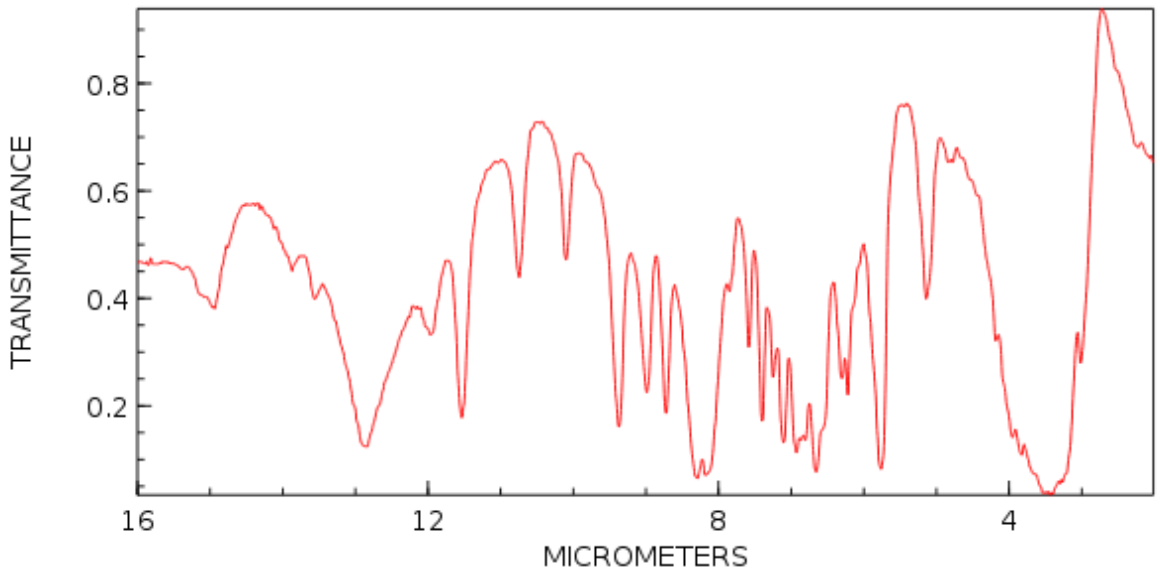


Fig 9 Typical FTIR spectrum of Uric Acid Stone standard

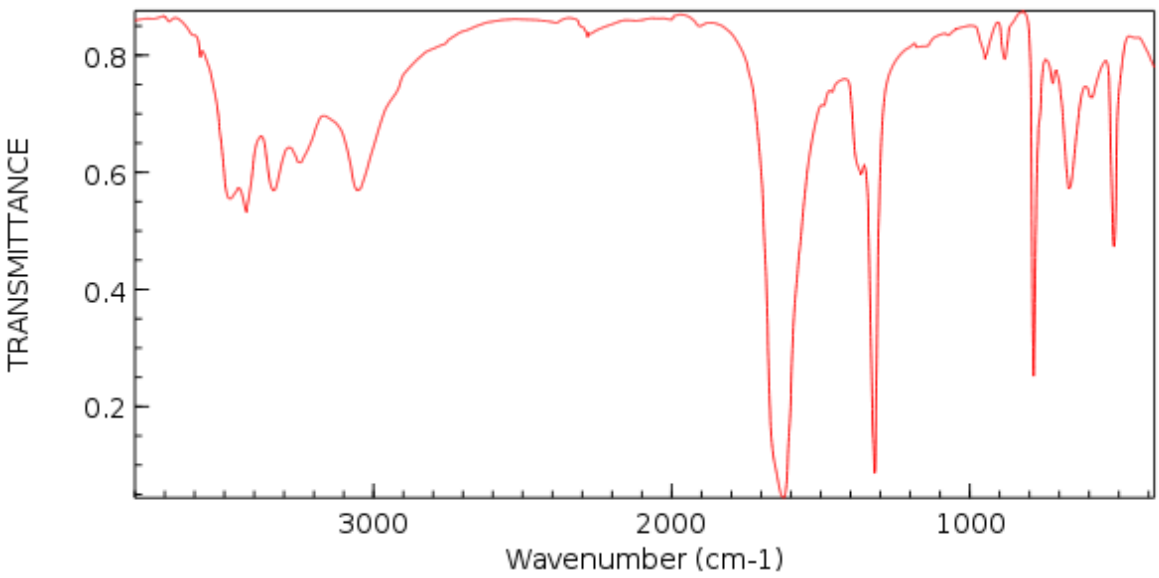


Fig 10 Typical FTIR spectrum of calcium oxalate standard

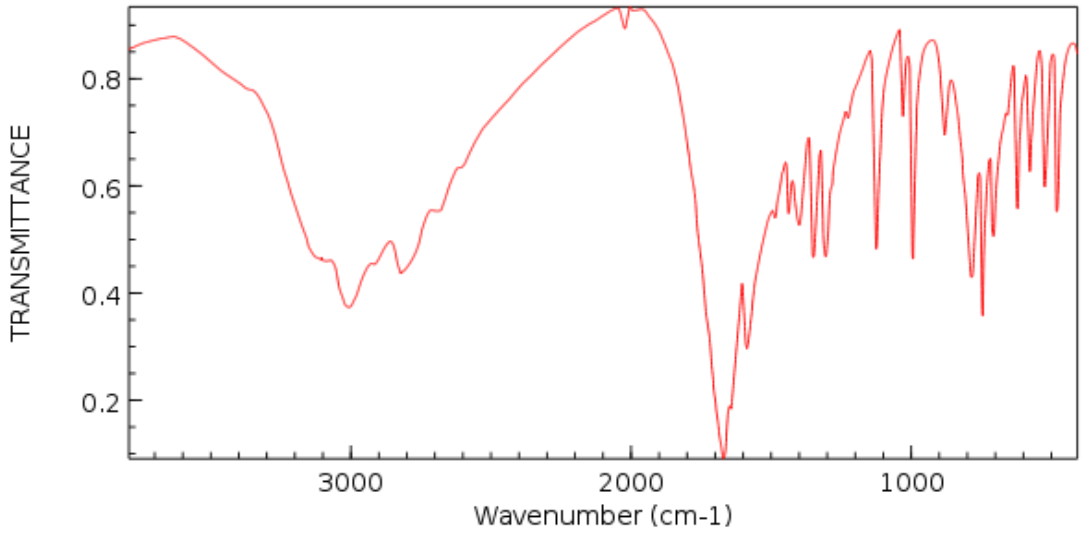


Fig 11 Typical FTIR spectrum of sycetine stone standard

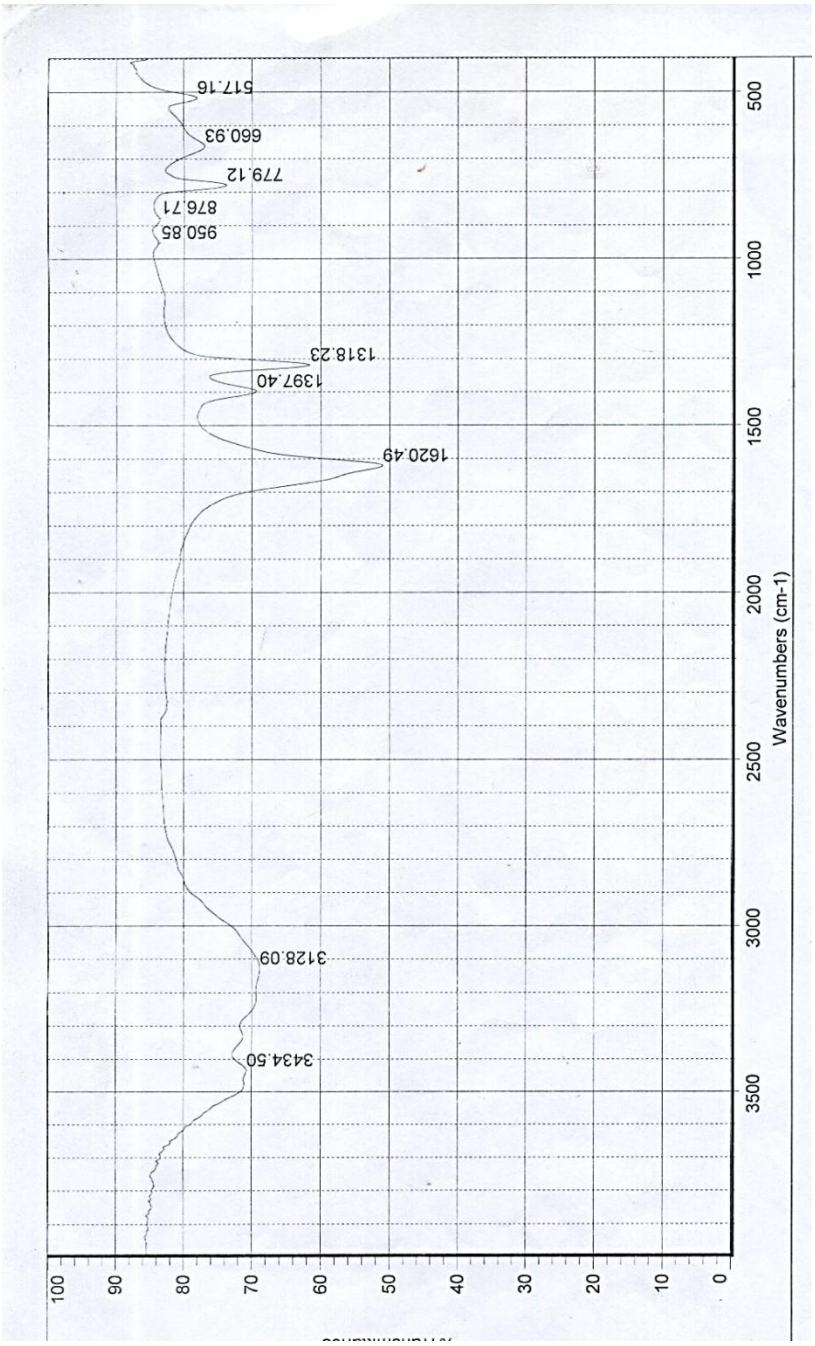


Fig 12 IR Spectrum of Sample 1

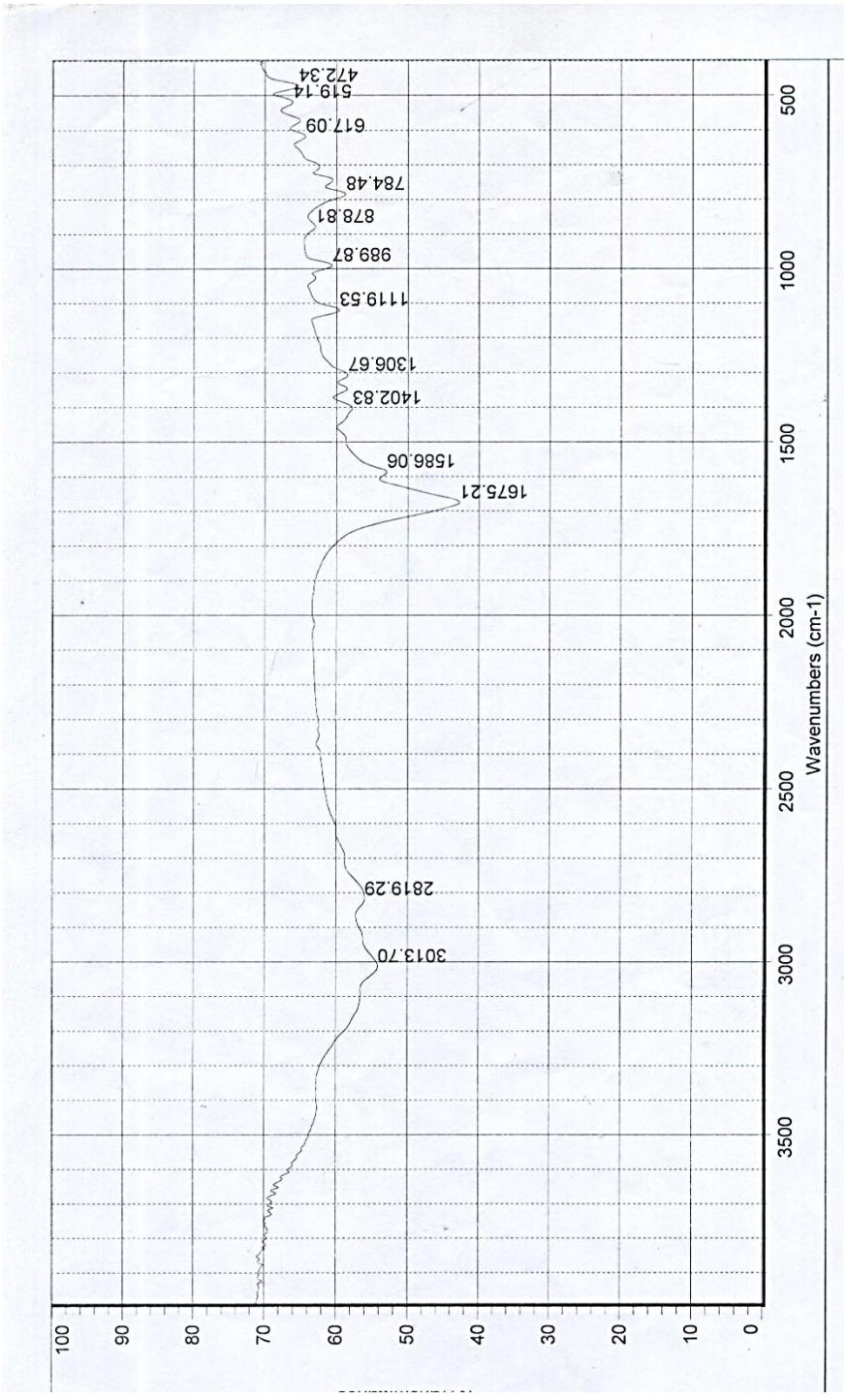


Fig 13 IR Spectrum of Sample 2

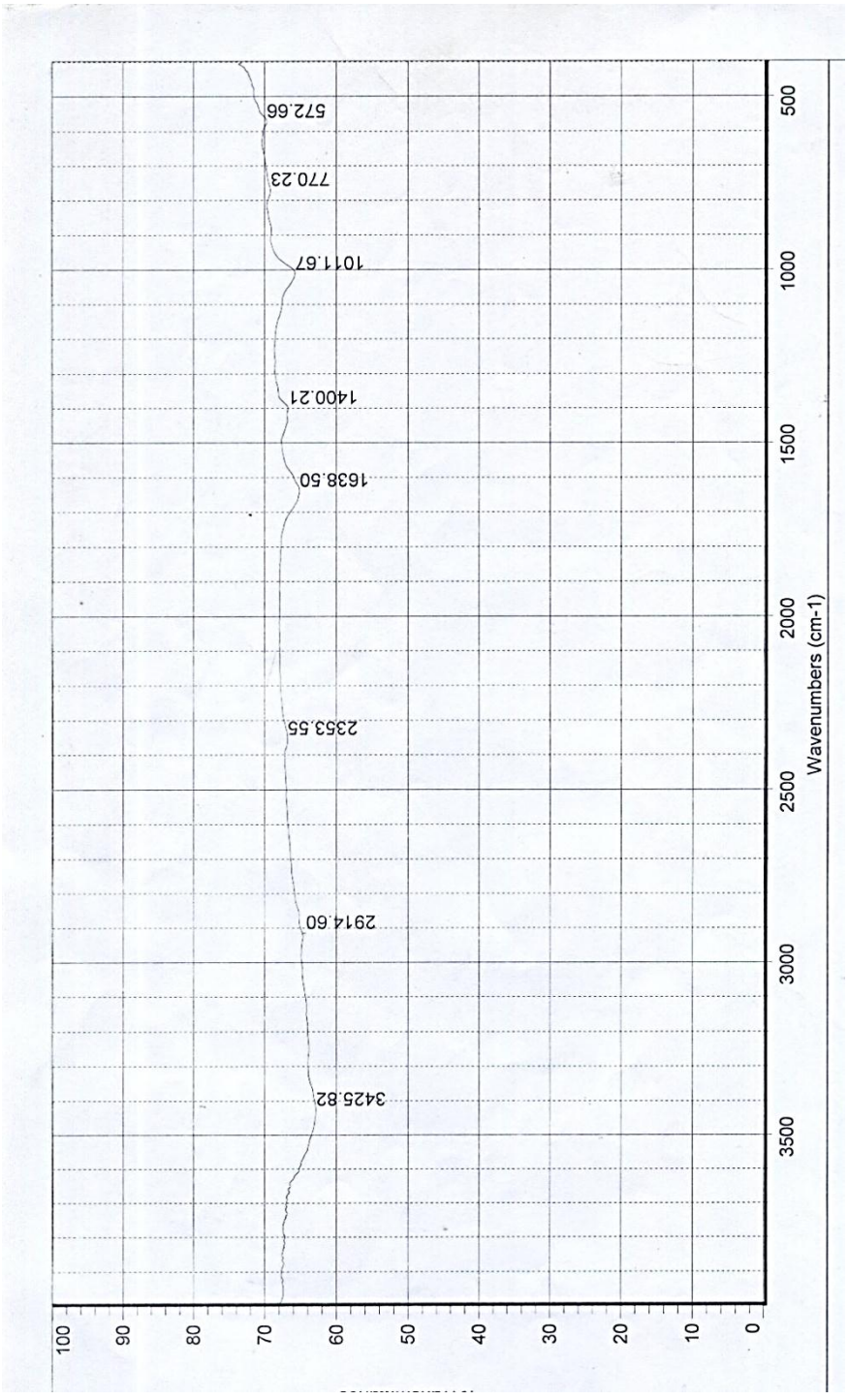


Fig 14 IR Spectrum of Sample 3

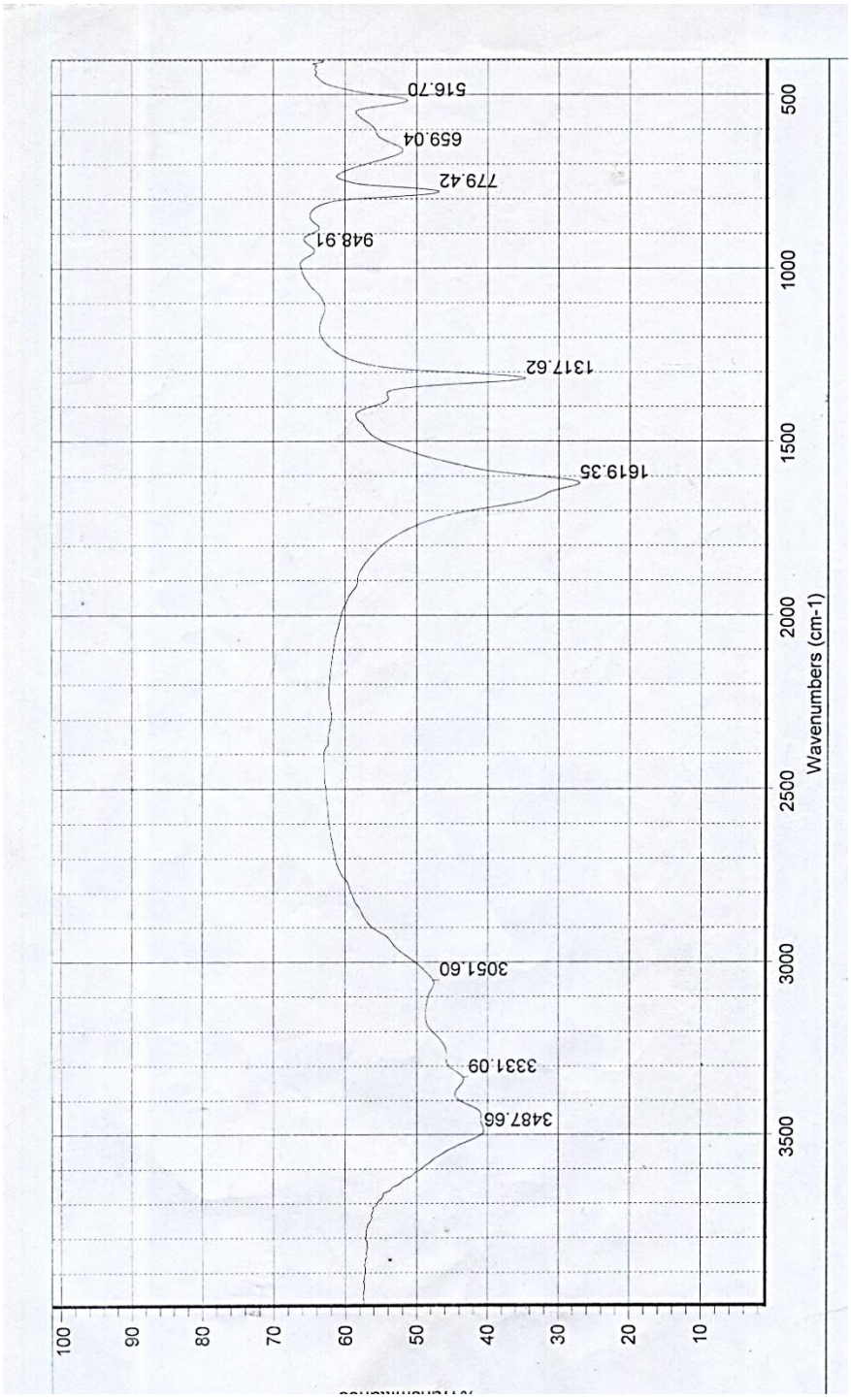


Fig 15 IR Spectrum of Sample 4

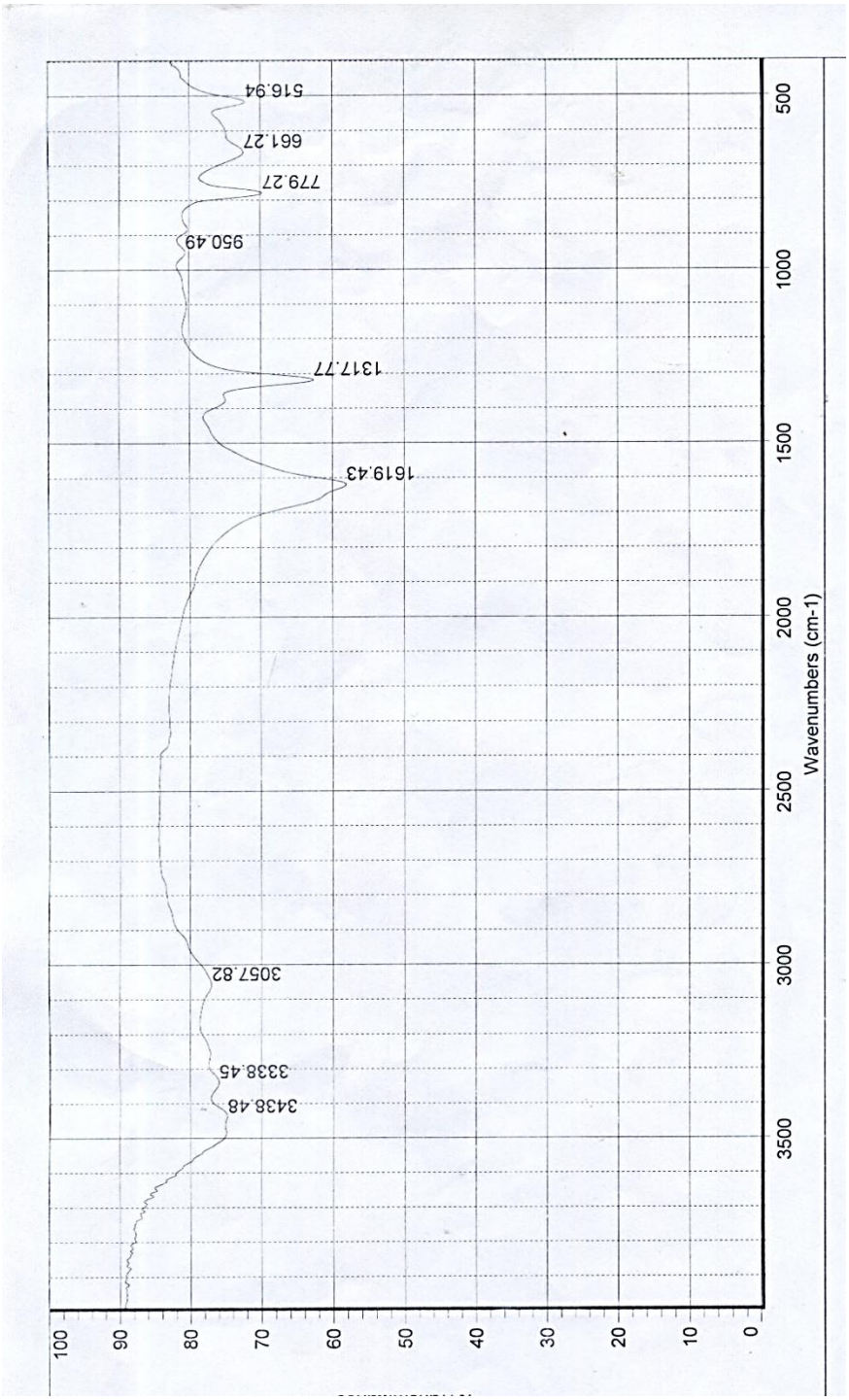


Fig 16 IR Spectrum of Sample 5

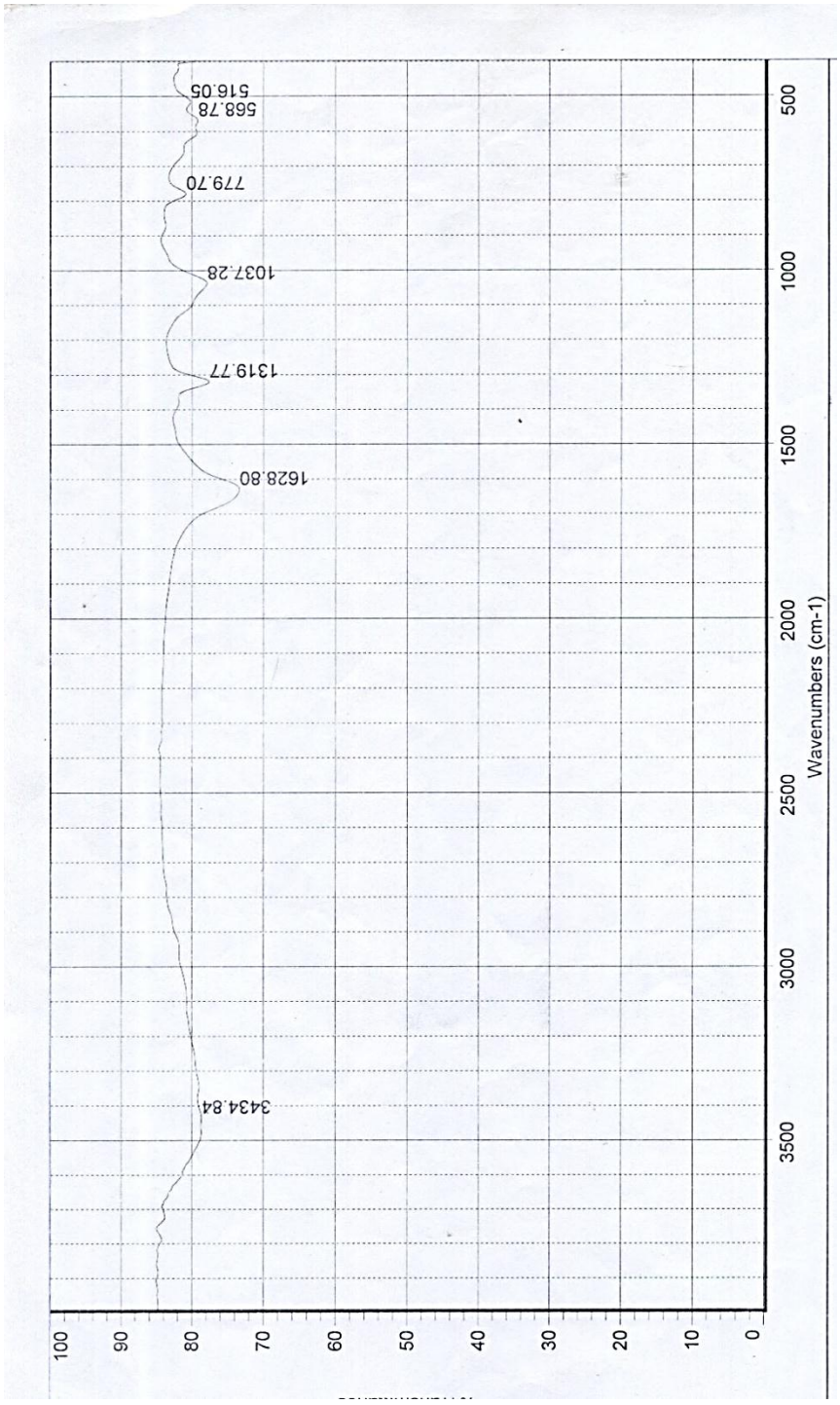


Fig 17 IR Spectrum of Sample 6

Typical FTIR spectra of four different standard stones are shown in figures 8 to 11 these were compared with the IR spectrum of 6 kidney stones samples figures12 to17 and found that samples 1, 2, 4 and 5were calcium oxalate and sturvite while sample 3 was composed of uric acid pure and sample 6was calcium oxalate pure

Naseem (2005) found that calcium oxalate stones mainly develop

3-3Ultra violet

Table 4 ultra violet spectra of kidney stones samples

Sample	absorbance	Wave length /nm
1	2.558	220
2	2.473	220
3	2.558	220
4	2.887	230
5	2.584	230
6	1.00	225

The wave length of all samples present in narrow range 220-230 nm and that attributed to presence of carbonyl group witch absorb radiation at 220 nm

(William Kemp 1994).

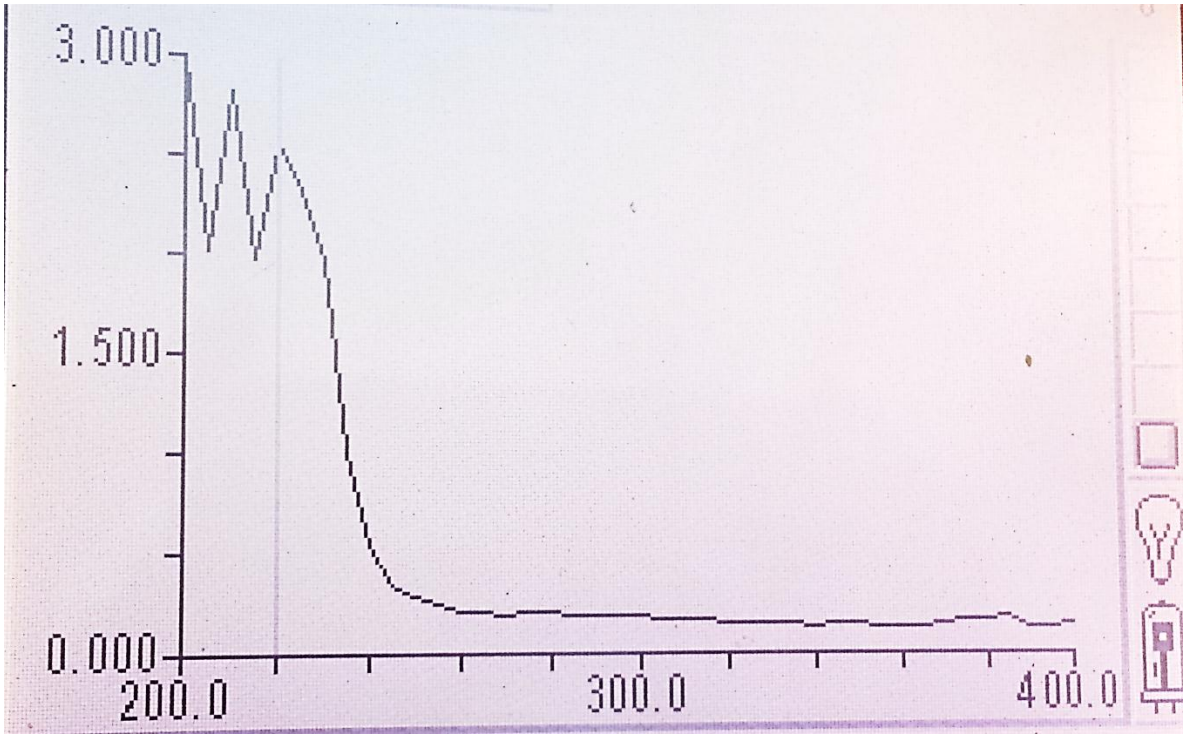


Fig 18 UV Spectrum of Sample 1

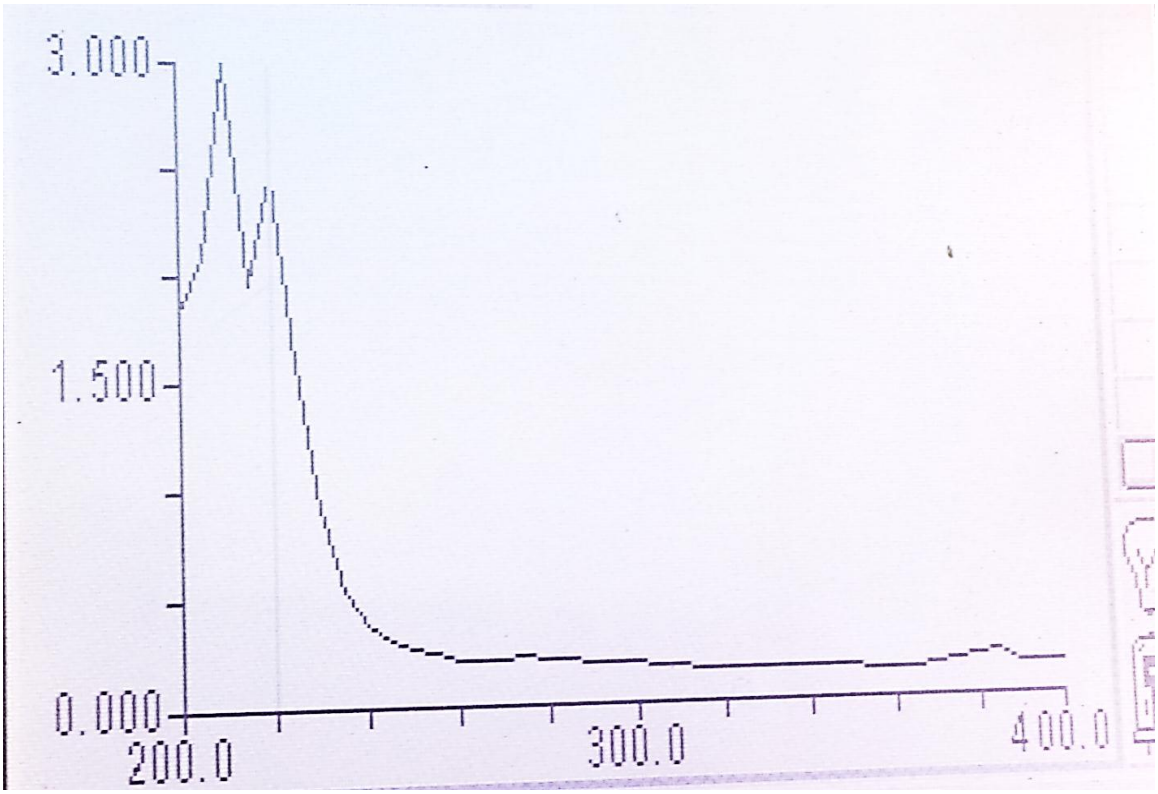


Fig 19 UV Spectrum of Sample 2

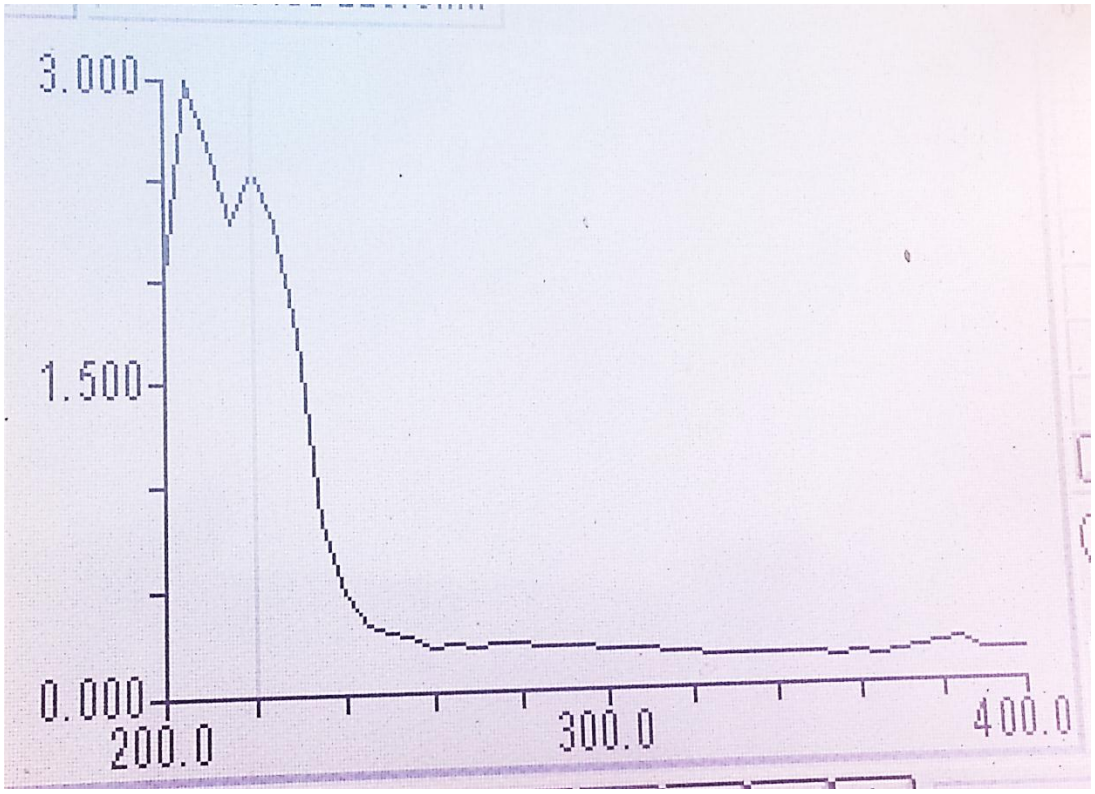


Fig 20 UV Spectrum of Sample 3

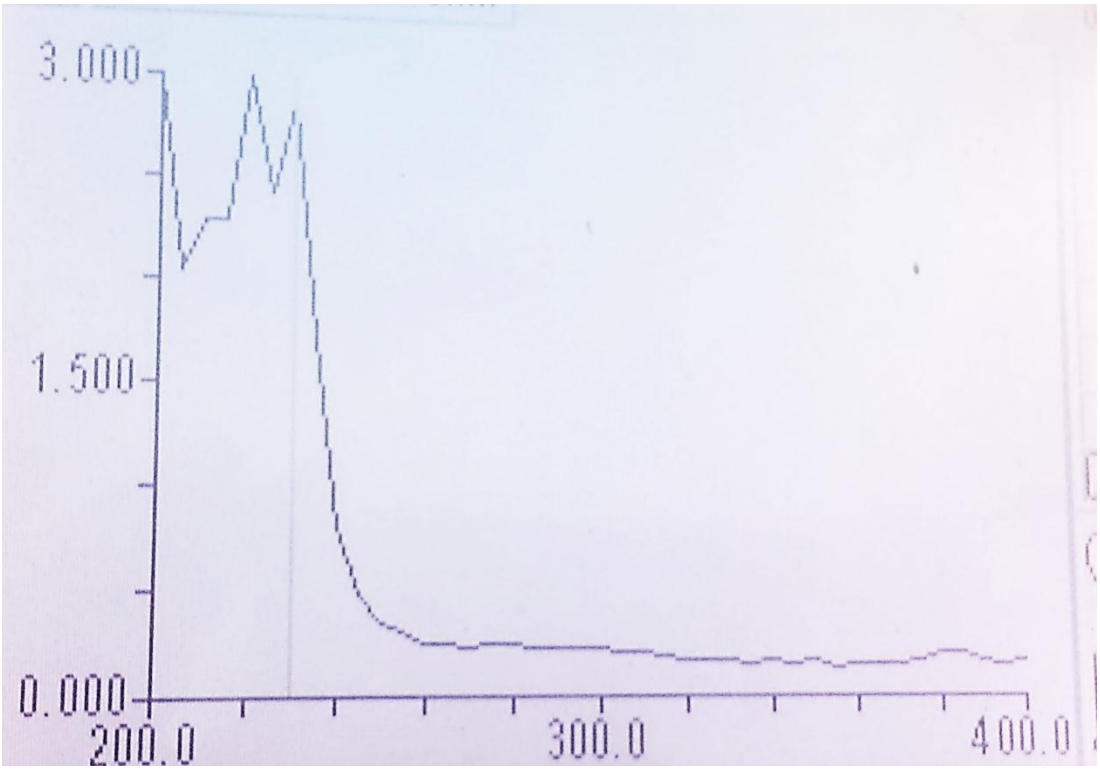


Fig 21 UV Spectrum of Sample 4

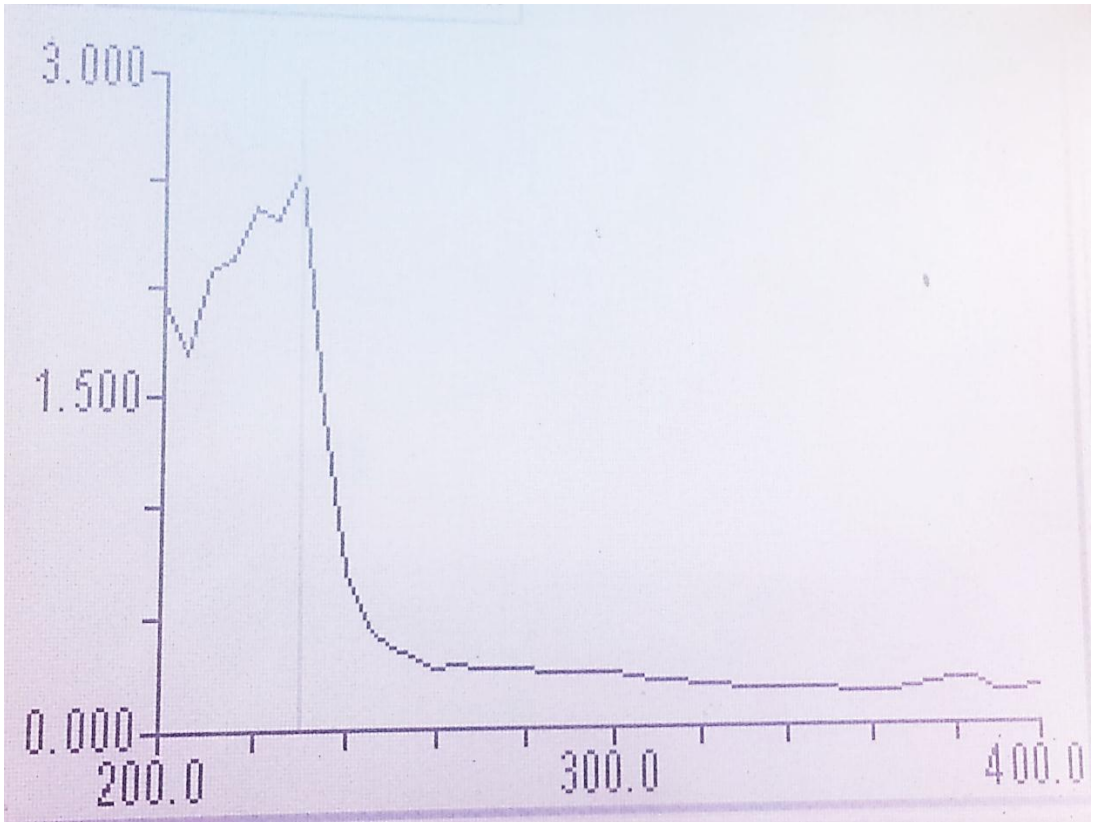


Fig 22 UV Spectrum of Sample 5

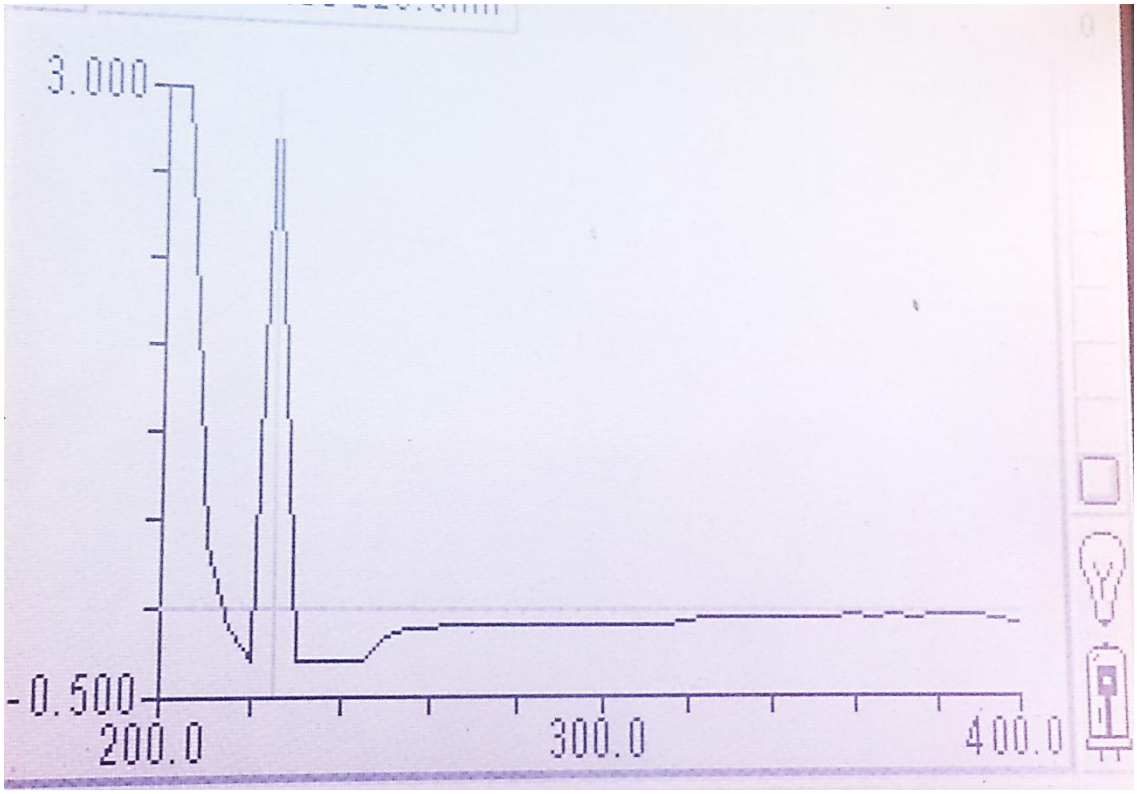


Fig 23 UV Spectrum of Sample 6

3-4 Statistical analysis results:-

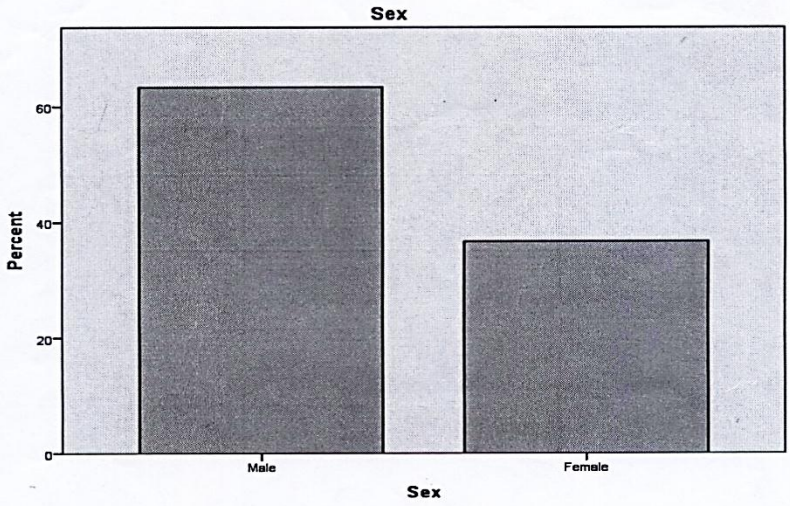
Table 5 statistical analysis of kidney stones patients from ShargeAlneel hospital

Sample no	Sex	Age	Resident	Place of stone
1	Male	46	med	Kidney
2	Female	28	Khartoum	Kidney
3	Male	48	Khartoum	Bladder
4	Female	25	east	Kidney
5	Female	18	west	Kidney
6	Female	65	unknown	Kidney
7	male	12	Khartoum	Bladder
8	female	50	unknown	Ureter
9	male	70	unknown	Bladder
10	male	43	med	Kidney
11	female	65	east	Kidney
12	male	27	med	Ureter
13	male	50	Khartoum	Ureter
14	male	76	Khartoum	Kidney
15	female	45	Khartoum	Kidney
16	female	55	east	Kidney
17	male	60	north	Ureter
18	female	26	med	Ureter
19	male	35	med	Bladder
20	male	34	med	Kidney
21	male	54	Khartoum	Kidney
22	female	68	Khartoum	Ureter
23	male	38	Khartoum	Ureter
24	male	33	Khartoum	Ureter
25	male	75	north	Bladder
26	male	54	east	Kidney
27	male	41	east	Kidney
28	female	70	South	Kidney
29	male	41	Khartoum	Kidney
30	male	20	South	Bladder

Statistical analysis :-
SPSS 21

Sex

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	19	63.3	63.3	63.3
	Female	11	36.7	36.7	100.0
	Total	30	100.0	100.0	



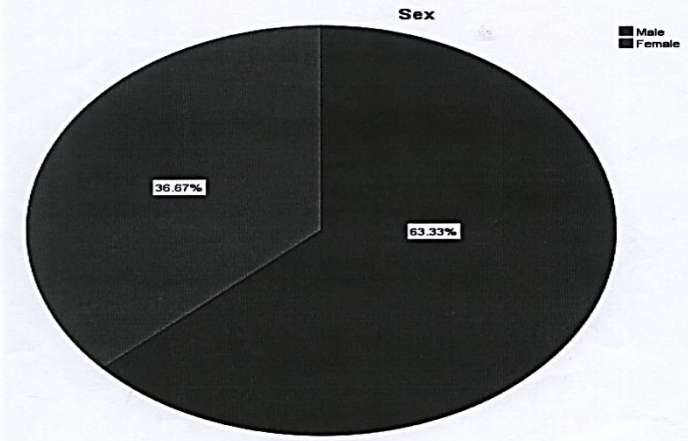
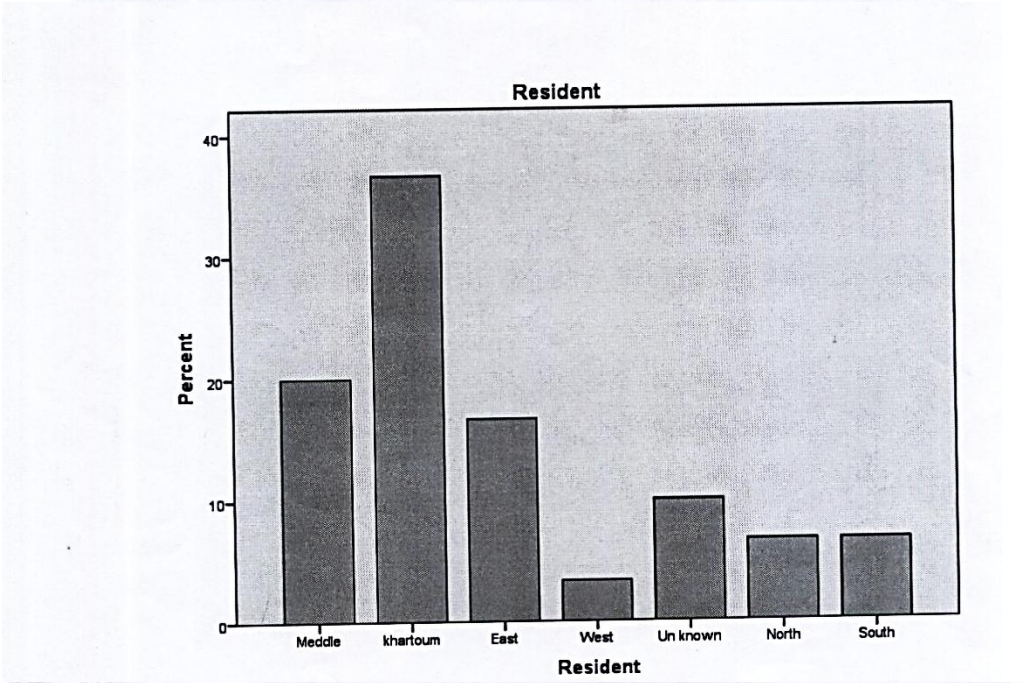


Fig 24 gender of patients

Resident

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Meddle	6	20.0	20.0	20.0
	khartoum	11	36.7	36.7	56.7
	East	5	16.7	16.7	73.3
	West	1	3.3	3.3	76.7
	Un known	3	10.0	10.0	86.7
	North	2	6.7	6.7	93.3
	South	2	6.7	6.7	100.0
	Total	30	100.0	100.0	



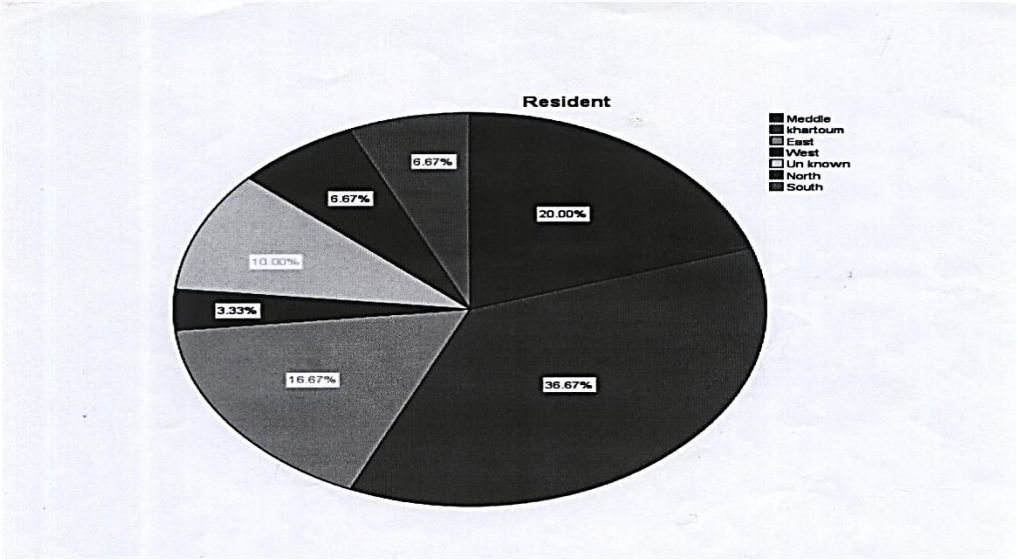


Fig 25 Patients Resident

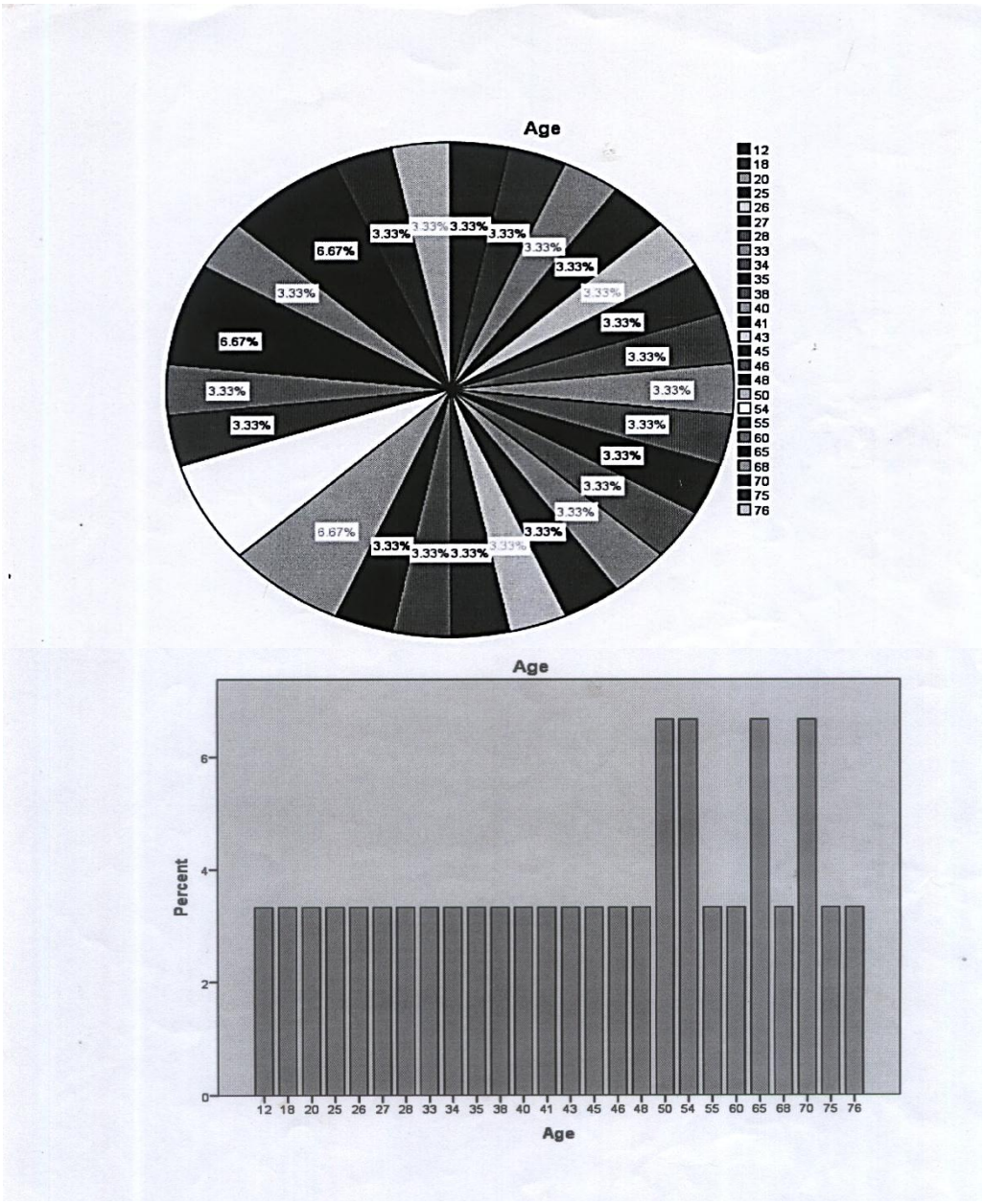
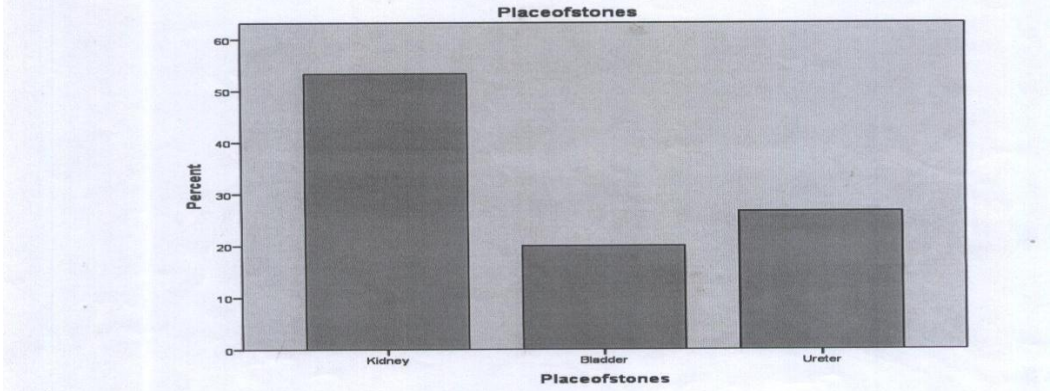


Fig 26 Ages of Patients

Placeofstones

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Kidney	16	53.3	53.3	53.3
	Bladder	6	20.0	20.0	73.3
	Ureter	8	26.7	26.7	100.0
	Total	30	100.0	100.0	



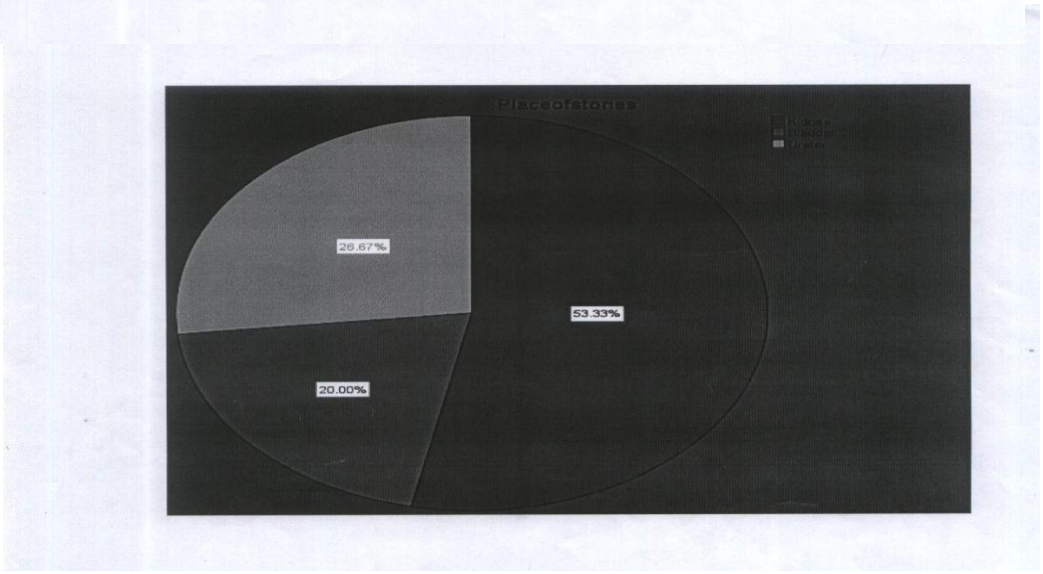


Fig 27 Place of stones

The statistics analysis of kidney stones collected from Shargealneel hospital revealed that kidney stones commonly occurred in age range from 18 to 70 years old SPSS analysis showed that they were it's commonly present in men rather than in women . Khartoum state resident are more susceptible to kidney stones than other residents; this can be related to a high population density. The location of stone in Sudanese patients found mainly in kidney.

Conclusion

Calcium oxalate was commonly found in Sudanese patients.

The mean elements presence in kidney stone have high concentration are calcium, Zinc and Iron in sample 5.

Mean age's stone formation from 18 to 70 years old.

Males are commonly more suceptible to stone formation than females.

There are four types of kidney stones, calcium oxalate, and uric acid, struvite and cystine, it's not responsive with IR spectroscopy because its resulting from a genetic disorder.

Recommendations:-

The patient must:

-Analyze the type of kidney stone

-Drink more fluids during the day

-Reduce foods that contain oxalate

-Eat less animal protein amounts

-Reduce coffee consumption

-Eat generally healthy diet as prevention is better than cure

- Treat water in areas where kidney disease is common

-Prevent and treat stones by natural products.

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2-Naseem AslamChanna ,AllahBux , aliMuhammedSoomoro and LubnaNoorani " Chemical Analysis of Kidney stoneby FTIR spectroscopy "

3-Raju K and C Rakkappan " Determination of chemical composition on Adult Kidney Stones _ A spectroscopy study" (2008)

4-Raymond KO "Kidney Stone Clinic" (2009)

5-Willam Kemp "Organic Spectroscopy third addition P147 (1994)

6-Saurin R.Shah , Manhai D.Desai , Lalit Panchal " classification of Renal Calculi" (2010) .

Appendices:-

sycteine stone

struvite stone

Uric Acid Stone

calcium oxalate stone