



Sudan University of Science & Technology
College of Graduate Studies



Chemical Characterization of Kidney Stone Samples from Some Sudanese Patients

توصيف كيميائي لعينات حصاوي الكلى من بعض المرضى السودانيين

A Thesis Submitted in Partial Fulfillment for the Requirements of M.Sc
Degree in Chemistry

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استهلال

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿قَالَ رَبِّ اشْحَبْ لِي صَدْرِي (25) وَيَسِّرْ لِي أَمْرِي (26) وَاحْلُلْ عُقْدَةً مِنْ لِسَانِي

(27) يَفْقَهُوا قَوْلِي (28)﴾

صدق الله العظيم

سورة طه الآيات (28-25)

Dedication

To the soul of my father, to my mother, to
my husband and children, to my brothers
and sisters.

Acknowledgement

All my thanks would go to Almighty Allah, for helping me to complete this work successfully.

I would like to thank my supervisor Dr. Omer Adam Gibla for his continuous encouragement, advice and close supervision.

My thanks would extend to the members of chemistry department in Sudan University of Science and Technology.

Thanks would also extend to all those who gave me help, encouragement or support.

Abstract

The aim of this study was to investigate the chemical composition of kidney stones obtained from some Sudanese patients. The samples were collected after surgical removal.

The samples were analyzed by FTIR and XRF spectroscopic techniques. Titrimetric analysis was carried for determination of oxalate content using standard potassium permanganate solution.

The IR analysis spectra of the samples showed the appearance of (C-H), (C=O), (O-C), (N-H), (C-C), (P-CH₂), (C=C), (P=O), (R-O), (P-O), (C-N), (O-H), (C=C), (C≡N), (OC-O), (P-O-C) . All samples showed (C=O) functional group occurrence.

X-Ray fluorescence analysis indicated the presence of P, Ca, Zn, Sr, Cu, Fe, Br and Pb.

Redox titration indicated that all the samples were dominated by high oxalate content, ranging from 22.88% to 62.48%.

المستخلص

هدفت هذه الدراسة لاستقصاء التكوين الكيميائي لعينات من حصاوي الكلى تم الحصول عليها من مرضى سودانيين بعد إزالتها جراحياً.

تم إجراء التحليل على عينات من حصاوي الكلى باستخدام تقنيتي الأشعة تحت الحمراء وفلورة الأشعة السينية.

كذلك تم تقدير نسبة الاوكسالات باستخدام محلول برمنجنات بوتاسيوم قياسي.

قياسات مطيافية الأشعة تحت الحمراء اظهرت وجود الزمر الوظيفية (C-H), (C=O), (O-C), (N-H), (C-C), (P-CH₂), (C=C), (P= O), (R-O), (P-O), (C-N), (O-H), (C= C), (C≡N), (OC-O), (P-O-C). كل العينات أظهرت وجود الزمرة الوظيفية (C=O).

التحليل عبر فلورة الأشعة السينية اظهر وجود العناصر Fe, Cu, Sr, Zn, Ca, P, Br, Pb .

معايير الاكسدة والاختزال اظهرت ان كل العينات تحتوي على نسبة عالية من الاوكسالات تتراوح بين 22.88% إلى 62.48%.

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Chapter one

Introduction

Introduction

1.1 Urinary system

1.1.1 Definition

The urinary system, or the renal system, consists of kidneys, ureters, bladder, and the urethra. Each kidney consists of millions of functional units called nephrons.

1.1.2 Structure

The urinary system refers to the structures that produce and conduct urine to the point of excretion. The human body normally has two paired kidneys, one on the left and one on the right. Urine is formed by nephrons, which is functional unit of the kidney, and then flows through a system of converging tubules called collecting ducts. The collecting ducts join together to form minor calyces, then major calyces, which ultimately join the pelvis of the kidney (renal pelvis). Urine flows from the renal pelvis into the ureter, a tube-like structure that carries the urine from the kidneys into the bladder.

During urination, urine stored in the bladder is discharged through the urethra. In males, the urethra begins at the internal urethral orifice in the trigone of the bladder, continues through the external urethral orifice, and then becomes the prostatic, membranous, bulbar, and penile urethra. Urine exits through the external urethral meatus. The female urethra is much shorter, beginning at the bladder neck and terminating in the vaginal vestibule (*Frederic L. et al., 2013*).

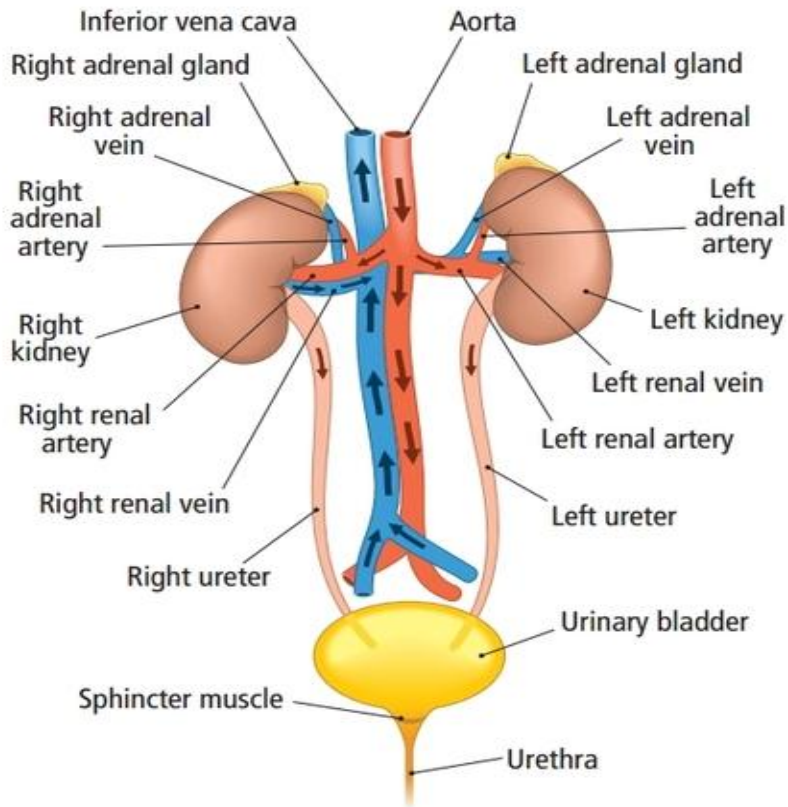


Figure (1.1): Urinary system

The role of the renal system is to eliminate wastes from the body, regulate blood volume and blood pressure, control levels of electrolytes and metabolites, and regulate blood PH. The kidneys have extensive blood supply via the renal arteries which leave the kidneys via the renal vein. Following filtration of blood and further processing, wastes in the form of urine exit the kidney via the ureters that propel urine towards the urinary bladder, where it is stored and subsequently expelled by urination.

Urine is formed in the kidneys through a filtration of blood. The urine is then passed through the ureters to the bladder, where it is stored. During urination, the urine is passed from the bladder through the urethra outside the body.

800–2,000 milliliters of urine are normally produced every day in a healthy human. This amount varies according to fluid intake and kidney function (*C. Dugdale, David 2011*).

1.2 Kidney stones

It is a solid piece of material which is formed in the kidneys from minerals in urine. It is also known as a renal calculus or nephrolith. Kidney stones normally leave the body in the urine stream, and a small stone may pass without causing symptoms. If stones grow to sufficient size usually at least 3 millimeters they can cause blockage of the ureter. This leads to pain,. Blockage of the ureter can cause decreased kidney function and dilation.

Most stones form due to combination of genetics and environmental factors. Risky factors include being overweight, certain foods, some medications, and not drinking enough fluids. Urinary stones are typically classified by their location in the kidney (nephrolithiasis), ureter (uretero lithiasis), or bladder (cysto lithiasis), or by their chemical composition (calcium-containing, struvite, uric acid, or other compounds) (*Frederic L. et al., 2013*).

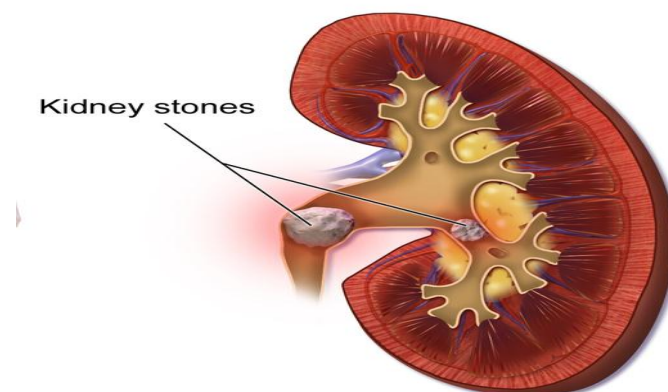


Figure (1.2): Position of stones in kidney

1.3 Historical Background

The existence of kidney stones was recorded thousands of years ago, and lithotomy for the removal of stones is one of the earliest known surgical procedures. In 1901, a stone was discovered in the pelvis of an ancient Egyptian mummy was dated to 4,800 BC. Medical from ancient Mesopotamia, India, China, Persia, Greece, and Rome all mentioned calculous disease. Part of the Hippocratic Oath suggests that there were practicing surgeons in ancient Greece to whom physicians were to defer for lithotomies. The Roman medical treatise *De Medicina* by Aulus Cornelius Celsus contained a description of lithotomy, and this work served as the basis for this procedure until the 18th century.

New techniques in lithotomy began to emerge starting in 1520, but the operation remained risky. After Henry Jacob Bigelow popularized the technique of litholapaxy, the mortality rate dropped from about 24% to 2.4%. However, other treatment techniques continued to produce a high level of mortality, especially among inexperienced urologists. In 1980, Dornier MedTech introduced extracorporeal shock wave lithotripsy for breaking up stones via acoustical pulses, and this technique has since come into widespread use (*Bard, et al., 2003*).

1.4 Importance of Kidneys

For anybody to work properly, it must contain just the right amount of water. One of the important jobs of the kidneys is to remove excess water from the body or to retain water when the body needs more.

Kidneys removing waste products and help to balance the body's minerals: Many substances in the blood and other body fluids must be kept at the correct level for the body to function properly. For example, sodium and potassium are minerals that come from food, the body needs these minerals for good health, but they must be kept at certain levels. When the kidneys are working properly, extra minerals,

such as sodium and potassium, leave the body in urine, the kidneys also help to adjust the levels of other minerals, such as calcium and phosphate which are important for bone strength, growth and other functions.

Kidneys help to remove waste products, such as urea and creatinine, from the body. Urea and other wastes are formed when the body breaks down protein, such as meat. Creatinine is a waste product of the muscles. As kidney function decreases, the levels of urea and creatinine in the blood increase. The creatinine level in blood is a very useful measure of kidney function. It is measured by a simple blood test.

1.4.1 Producing hormones

Normal kidneys also produce or form important chemicals in the body known as hormones. Hormones circulate in the bloodstream like “messengers” and regulate blood pressure, red blood cell production and calcium balance.

Table (1.1): Normal ranges of some blood constituents

Constituent	Range
Urea	1.8 - 8.2 mmol/L
Potassium	3.5 - 5.0 mmol/L
Phosphate	0.8 - 1.4 mmol/L
Calcium	2.0 - 2.6 mmol/L
Creatinine	60 - 110 pmol/L (females) 70 - 120 pmol/L (males)
Hemoglobin	120 - 140 g/L (females) 140 - 160 g/L (males)
GFR*	90 - 120 ml/min (1.5 - 2.0 ml/sec)

The amount of each substance in blood can be measured by several ways: for eg; milli moles, micro mols or grams per litre of blood (g/L). Table (1.1) shows the range of the normal levels of various substances in blood of a healthy person.

1.5 Kidney Stones and Kidney Failure

Kidney stones in some cases can actually result in a “dead” kidney or kidney failure. For this doesn’t happen very often and it may takes a long time to occur, providing the opportunity for treatment before permanent damage occurs.

Kidney stones can cause kidney damage in two primary ways:

- a. An untreated obstructing stone that causes persistent severe blockage instead of successfully passing can eventually cause atrophy in a kidney, resulting in a dilated, thinned out kidney with minimal function. Most stones are associated with significant amounts of pain, most patients will seek treatment long before permanent damage can occur. However, in cases where patients have “silent” stones that cause little or no pain, long term obstruction can occasionally lead to kidney damage. With no symptoms to warn them, these patients often go months to years before a stone is diagnosed.
- b. Infection related stones, usually composed of struvite and sometimes presenting as a complete “staghorn” can lead to ongoing chronic urinary tract infections that cause damage slowly through inflammation and scarring of the kidney tissue.

1.6 Causes of Kidney Stones formation

Dehydration due to low fluid intake is a major factor in stone formation. High dietary intake of animal protein, sodium, refined sugars, fructose and high fructose corn syrup, oxalate, grapefruit juice, and apple juice may increase the risk of kidney stone formation.

A major risk factor for kidney stones is constant low urine volume. Low urine volume may come from dehydration or loss of body fluids from hard exercise, working or living in a hot place, or not drinking enough fluids. When urine volume is low, urine is concentrated and become dark in color. Concentrated urine means there is less fluid to keep salts dissolved. Increasing fluid intake will dilute the salts in urine and reduce risk of stones forming (*Peter Crosta 2017*).

Adults who form stones should drink enough fluid to produce at least 2.5 liters of urine every day. On average, this will take about 3 liters of fluid intake per day. Water is likely the best fluid to drink.

Diet can also support the chance of forming a stone. One of the more common causes of calcium kidney stones is high levels of calcium in the urine. High urine calcium levels may be due to the way of low body handles calcium. It is not always due to how much calcium we eat. Lowering the amount of calcium in the diet rarely stops stones from forming. Studies have shown that restricting dietary calcium can be bad for bone health and may increase kidney stone risk. Health care providers usually do not tell people to limit dietary calcium in order to lower urine calcium. But calcium intake should not be too high (*Peter Crosta 2017*)

Instead of lowering dietary calcium intake, the health care provider may try to reduce the calcium level by decreasing sodium (salt) intake. Too much salt in the diet is a risk factor for calcium stones. This is because too much salt is passing into the urine, keeping calcium from being reabsorbed from the urine and into the blood. Reducing salt in the diet lowers urine calcium, making it less likely for calcium stones to form.

Because oxalate is a component of the most common type of kidney stone, eating foods rich in oxalate can raise the risk of forming calcium oxalate stone).

Diet high in animal protein, such as beef, fish, chicken and pork, can raise the acid levels in the body and in the urine. High acid levels make it easier for calcium oxalate and uric acid stones to form. The breakdown of meat into uric acid also raises the chance that both calcium and uric acid stones will form (*Emamian, et al., 1993*)

1.6.1 Bowel Conditions and obesity

Certain bowel conditions that cause diarrhea, such as chronic disease or ulcerative colitis or surgeries such as gastric bypass surgery, can raise the risk of forming calcium oxalate kidney stones. Diarrhea may result in loss of large amounts of fluid from the body, lowering urine volume. Human body may also absorb excessive oxalate from the intestine, resulting in more oxalate in urine. Both low urine volume and high levels of urine oxalate can help to cause calcium oxalate kidney stone formation.

Obesity is a risk factor for stones. Obesity may change the acid levels in the urine, leading to stone formation.

1.6.2 Medical conditions

Some medical conditions increase the risk of kidney stones. Abnormal growth of one or more of the parathyroid glands, which control calcium metabolism, can cause high calcium levels in the blood and urine. This can lead to kidney stones. Another condition called distal renal tubular acidosis, in which there is acid build-up in the body, can raise the risk of calcium phosphate, kidney stones.

Some rare, inherited disorders can also make certain types of stones more likely. Examples include cystinuria, which is too much of the amino acid cystine in the urine, and primary hyper oxaluria, in which the liver makes too much oxalate (*Walter F. et al., 2004*).

1.6.3 Medications and Family History

Some medications, such as calcium and vitamin C supplements, may increase the risk of forming stones. We may need to tell the health care provider all the medications and supplements we take, as these could affect the risk of stone formation.

The chance of having kidney stones is much higher if there is a family history of stones, such as a parent or sibling (*Nielsen, et al., 1995*).

1.7 Children Kidney Stones

Although kidney stones do not often occur in children, the incidence is increasing. These stones are in the kidney in two thirds of reported cases, and in the ureter in the remaining cases. Older children are at greater risk independent of age and sex. As with adults, most pediatric kidney stones are predominantly composed of calcium oxalate; struvite and calcium phosphate stones are less common. Calcium oxalate stones in children are associated with high amounts of calcium, oxalate, and magnesium in acidic urine.

1.8 Types of kidney stones

There are four basic kinds known as calcium stones.

Kidney stones may contain various combinations of chemicals.

Calcium stones, Uric acid stones, Struvite stones, and Cystine stones.

The stones are formed from the chemicals usually found in the urine such as uric acid, phosphorus, calcium, and oxalic acid or oxalate. They may vary in consistency from grit, sand, and gravel. Stones may form and grow because of the increasing concentration of a particular substance. The phosphate stone being most common in the presence of infection.

The most common type of stones contain calcium in combinations with either oxalate or phosphate are part of person's normal diet and make up important parts of the body such as bones and muscles. About 90 percent of all stones contain calcium as the chief constituent.

About 80 percent of all stones is calcium oxalate stones. A less common type of stones is caused by infection in the urinary tract, this type of stone is called a struvite or infection stones. Uric acid stones are a bit less common, and cystine stones are rare.

1.8.1 Calcium stones

There are two types of calcium stones; calcium oxalate and calcium phosphate. Calcium oxalate is by far the most common type. Some people have too much calcium in their urine, raising their risk of calcium stones. Even with normal amounts of calcium in the urine, calcium stones may form for other reasons.

1.8.2 Uric acid stones

Uric acid is a waste product that comes from chemical changes in the body. Uric acid crystals do not dissolve well in acidic urine and instead will form a uric acid stone. Having acidic urine may come from, being overweight, chronic diarrhea, type 2 diabetes (high blood sugar), gout, a diet that is high in animal protein and low in fruits and vegetables.

1.8.3 Struvite stones

Struvite stones are not common types of stones. They stones are related to chronic urinary tract infections. Some bacteria make the urine less acidic and more basic or alkaline. Magnesium ammonium phosphate (struvite stones) form in alkaline urine. These are often large, with branches, and they often grow very fast.

People who get chronic UTIs, such as those with long-term tubes in their kidneys or bladders, or people with poor bladder emptying due to neurologic disorders are at the highest risk for developing such stones.

1.8.4 Cystine stones

Cystine is an amino acid found in certain foods; it is one of the building blocks of protein. Cystinuria (form cystine in the urine) is a rare, inherited metabolic disorder. It is when the kidneys do not reabsorb cystine from the urine. When high amounts of cystine are in the urine, it causes stones formation. Cystine stones often start to form in childhood.

1.9 Kidney Stones Drugs

To prevent stones formation, the type of stone and the urine abnormalities will help decide when there is medicine need, and which medicine is best.

1.9.1 Thiazides diuretics

These are for patients who have calcium stones and high levels of calcium in their urine. Thiazides lower urine calcium by helping the kidney take calcium out of the urine and release it back in the blood stream. Persons who take thiazides, may need to limit how much salt they take in, as these medications work best when urine sodium is low.

1.9. 2 Potassium citrate

This is for patients with calcium stones and low urinary citrate, and for those with uric acid and cystine stones. Potassium citrate makes the urine less acidic or more alkaline. This helps to prevent cystine and uric acid stones. It also raises the citrate level in the urine, helping to prevent calcium stones.

1.9.3 Allopurinol

This is frequently prescribed for gout, which is caused by high uric acid in the blood. Allopurinol not only lowers the level of uric acid in the blood but also in the urine, so it may also be prescribed to help prevent calcium and uric acid stones.

1.9.4 Acetohydroxamic acid (AHA)

Is for patients who produce struvite or infection stones. These stones form because of repeated urinary tract infections (UTI). AHA makes the urine unfavorable for struvite stones to form. The best way to prevent struvite stones is to prevent repeated UTIs caused by specific types of bacteria and to completely remove the stones with surgery.

1.9.5 Cystine-binding thiol drugs

These types of drugs are used only for patients who form cystine stones. These medications (d-penicillamine or tiopronin) bind to cystine in the urine and form a compound that is less likely than cystine to crystallize in the urine. This drug is used when other measures fail, such as raising fluid intake, reducing salt intake or using potassium citrate.

1.9.6 Vitamin supplements

Vitamins should be used carefully as some can increase the risk of forming kidney stones. The health care provider and a dietitian may be good sources of information about over-the-counter nutritional supplements.

1.10 Medical Recommendations (prevention)

Once the health care provider finds out why are the stones forming, will give tips on how to prevent them. This may include changing diet and taking certain medications. There is no diet for preventing kidney stones. There are dietary

changes that you can make to stop stones from continuing to form. Below are some tips (**Diet Changes**)

a. Is to drink enough fluids each day

If there is not enough urine produced, health care provider will recommend drinking at least 3 liters of liquid each day. This equals about 3 quarts (about ten 10-ounce glasses). This is a great way to lower the risk of forming new stones. It is recommended to drink more to replace the lost fluid in case of sweat from exercise or in hot weather. All fluids count toward the fluid intake, but it's best to drink mostly no-calorie or low-calorie drinks. This may mean limiting sugar-sweetened or alcoholic drinks.

b. Reduction of salt in the diet

This tip is for people with high sodium intake and high urine calcium or cystine. Sodium can cause both urine calcium and cystine to be too high. The health care provider may advise to avoid foods that have a lot of salt.

c. Eating the recommended amount of calcium

Taking calcium supplements make sure you aren't getting too much calcium. On the other hand make sure you aren't getting too little calcium either. Talk with the health care provider or dietitian about whether you need supplements.

Usually getting enough calcium from the diet without supplements if eating three-to-four servings of calcium-rich food. Many foods and beverages have calcium in them. Some foods and beverages that might be easy to include on a daily basis with meals are:

- i. Eat foods with low oxalate levels.
- ii. Eat less meat.

1.11 Previous studies

Fredric L Coe et al., (2005), reported that about 5% of American women and 12% of men will develop a kidney stone at some time in their life, and prevalence has been rising in both sexes.

Approximately 80% of stones are composed of calcium oxalate (CaOx) and calcium phosphate (Cap) 10% of struvite (magnesium ammonium phosphate produced during infection with bacteria that possess the enzyme urease, 9% of uric acid; and the remaining 1% are composed of cystine or ammonium acid urate or are diagnosed as drug-related stones. Stones ultimately arise because of an unwanted phase change of these substances from liquid to solid state. Here we focus on the mechanisms of pathogenesis involved in CaOx, Cap, UA, and cystine stone formation, including recent developments in our understanding of related changes in human kidney tissue and of underlying genetic causes, in addition to current therapeutics.

In a study conducted by Abboud (2007) it was reported that, due to the increase in the number of urinary calculi disease cases in Jordan, stone samples were collected from patients from various Jordanian hospitals. The study concentrates on the effect of trace metals in patients of urinary calculi. Trace metals were detected in 110 urinary calculi samples using X-ray fluorescence (XRF) and atomic absorption spectroscopy (AAS) techniques. Of the calculi examined, 21 were pure calcium oxalate (CaOx), 29 were mixed calcium oxalate/uric acid, 23 were mixed calcium oxalate/phosphate (apatite), 25 were phosphate calculi (apatite/struvite), five were mixed calcium oxalate monohydrate/struvite, four were urate calculi (mixed ammonium acidurate/sodium acid urate) and three were pure cystine calculi. The concentration measurement of Ca and other metals levels has been found useful in understanding the mechanism of stone formation and in evaluating pathological

factors. It has been found that Ca is the main constituent of the urinary calculi, especially those stones composed of calcium oxalate and calcium phosphate. The concentrations of the determine minerals were found to be (Ca 48.18%, Na 1.56%, K 0.9%, Mg3.08 %, Fe 1.17%, Al 0.49%, Zn 0.7%, Cu 0.19%, Mn 0.029%, P 10.35%, S 1.88%, Sr 0.306%,Mo 0.2%, Cr 0.146%, Co 0.05%,Ni 0.014%). In conclusion, metals concentration in Jordanian patient's urinary calculi samples was higher²⁹than its equivalents of other patients'. It has been noted that there is no concentration of toxic trace elements (like Li, V, Pb, Cd, and As). Some heavy metals, however, were detected Mo, Cr, Co and Ni as traces. Phosphorus (P) and Sulfur (S) ions are present in few calculi stones as traces.

Studies of renal stone disease in Thailand, were carried by Eimorn et al., (2002) using proton magnetic resonance spectroscopy, indicated abnormal urinary aggregator and inhibitor composition among farmers with excessive sweat loss. The above study compared the proton MR spectra obtained from the kidneys of 32 proven cases of RSD (aged 38 to 65 yrs) with nine age matched normal control subjects. They used the STEAM sequence with TE _15 ms and TR _ 2000 ms. The spectra at 3.25, 3.6 and 3.9 ppm were analyzed. The results showed a correlation between the three peaks (p _0.001), however, there was no significant difference between the RSD group and the normal control subjects. Therefore, they concluded that there was no overloading of these osmolytes among the renal stone patients.

Selvaraju, R., et al., (2012) used FT-IR spectroscopy technique to analyze the organic and and inorganic constituent of human urinary stones. The FT-IR results indicate that stones have different composition, i.e., namely calcium oxalate, calcium phosphate, carbonate apatite and magnesium ammonium phosphate and uric acid. From the spectral and powder X-ray³⁰diffraction pattern, the chemical constituents of urinary stones were identified. The quantitative estimations of

calcium oxalate monohydrate (COM) 1620 cm^{-1} , calcium phosphate (apatite) 1037 cm^{-1} , magnesium ammonium phosphate (struvite) 1010 cm^{-1} , calcium carbonate 1460 cm^{-1} and uric acid 1441 cm^{-1} were calculated using particular peaks of FT-IR studies. The study reveals that calcium oxalate monohydrate and calcium phosphate type urinary stones were predominant whereas magnesium ammonium phosphate are in moderate level, and calcium carbonate and uric acid are in low. Calcium phosphate is found in all the stones and calcium oxalate monohydrate is found to be higher. Quantitative analyses of urinary stones show that calcium oxalate monohydrate (40%), apatite (30%), magnesium ammonium phosphate (23%) and uric acid (7%) are present in all the urinary stone samples. Such the diet is affecting the uric acid levels.

Israhga El toom Abdalla Hussein (Sudan – 2016) reported that kidney stones are the third most common urological disease affecting both males and females worldwide. Environmental factors, especially diet play an important role in expression of the tendency to stone formation. The present study was aimed to analyze the kidney stones (collected from 20-sudanese patients), and some dietary factors (black tea, tap-water and table salt).

All samples were analyzed by inductively coupled plasma mass spectrometry Optical Emission Spectrometer (ICPOES), X-ray fluorescence, FTIR, and Ultra violet spectrometer. The results gave indication for the type of stone. Some samples were calcium oxalate stones, others calcium phosphate stones, and uric acid stones. The study of tap water, black tea and sodium chloride affected on kidney stones formation. The results of elements concentration on different kidney stone samples gave indication to the concentration of element, the content of elements Ba, Cu, Fe, K, Mg, Mn, Na, P and Se is high in dietary factors, probably that reason indicates the kidney stone formation.

Ishraga's study (2016) has also shown that in both groups of males and females there was an increase in elements content as the age increase.

The aim of this study is:

- i- To identify the main chemical composition of kidney stone samples by XRF technique for determination of the main elements in each sample.
- ii- To determine the functional groups to differentiate between the kidney stone types using (IR) analysis.
- iii- To determine oxalate content of samples.

Chapter Two

Materials and Methods

Materials and Methods

2.1 Samples collection

Kidney stone samples were obtained from five Sudanese patients after surgical removal. The patients are four males and one female of different ages and from different areas. Table (2-1) showed the most available drinking water sources.

Table (2.1): Sample sources

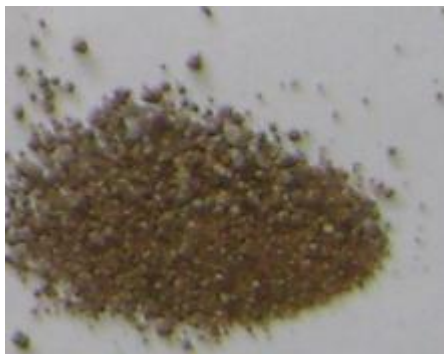
Sample	Gender	Age	Area	Drinking water sources
1	Male	25	Alhasaheha	Wells
2	Male	33	Atbara	Rivers and wells
3	Male	30	Kadogli	Groundwater and valleys
4	Male	35	Khartoum	Rivers and wells
5	female	45	Khartoum	Rivers and wells



Sample (1)



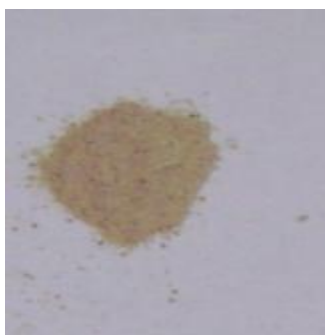
Sample (2)



Sample (3)



Sample (4)



Sample (5)

Figure (2.1): Samples Pictures

2.2 Chemicals

- Hydrochloric Acid (36 -38%), Wt. per ml at 200C 1.18
- Nitric Acid (69-72%), Wt. per ml at 200C 1.41
- Oxalic acid.
- Potassium permanganate.
- Potassium bromide

2.3 Instruments

2.3.1 Infrared spectrophotometer

(Shimadza-FTIR 8400s) for KBr pellet 0.195

2.3.2 XRF

Model Canberra series 35 plus, with Cd ¹⁰⁹ source and silicon detector, designed and manufactured in USA.

2.4 Methods of Analysis

2.4.1 Sample preparation for oxalate analysis

About 0.5 grams of each sample was accurately weighed and dissolved in aquaregia, with continues, stirring until complete dissolving. Each solution was filtered, quantitatively transferred to a (100ml) volumetric flask and the volume was completed to the mark with distilled water.

2.4.2 Determination of oxalate content

5 ml of each sample solution was transferred to a titration flask. 3ml of concentrated sulphuric acid was added and the mixture was heated on a hot plate to 60°C. The hot mixture was then titrated against potassium permanganate solution (0.02 M). The oxalate content was then calculated for each sample.

2.4.3 Determination of elements by XRF

The samples were first crushed into fine powder and then pressed into pellets using 15 ton pressing machine.

The diameter of each pellet was about (2.5 cm) and the mass about 1 g

The pellets were introduced to the XRF spectrometer.

The spectra were then analyzed.

2.4.4 Functional groups Determination

0.195 g of dried potassium bromide was weighed and (0.2) g of the sample was added. The mixture was homogenized for 2 minutes, and then pressed into disk and introduced to the (FTIR) instrument.

Chapter Three

Results and Discussion

3-Results and Discussion

Table 3.1 Determination of oxalate content

Sample No	Oxalate C ₂ O ₄ %	CaC ₂ O ₄
S ₁	61.6.6%	89%
S ₂	22.88%	33.28%
S ₃	39.6%	57%
S ₄	62.48%	90.88%
S ₅	43.12%	62.72%

All the samples showed a significant amount of oxalate range from 22.88% as a lower value and (62.48%) as a highest.

Table 3.2 XRF results

Sample number						
Elements	Unit	1	2	3	4	5
P	ppm mg/kg	9.82×10^{-2}	1.35×10^{-1}	1.8×10^{-1}	2.94×10^{-1}	9.82×10^{-2}
Ca		4.08×10^{-2}	6.74×10^{-2}	4.08×10^{-3}	4.17×10^{-3}	4.08×10^{-2}
Cu		9.49×10^{-7}	3.69×10^{-7}	2.2×10^{-6}	2.36×10^{-6}	9.49×10^{-7}
Zn		5.79×10^{-6}	4.05×10^{-5}	6.18×10^{-6}	1.45×10^{-5}	5.79×10^{-6}
Sr		4.09×10^{-5}	1.22×10^{-5}	8.8×10^{-6}	9.8×10^{-7}	4.09×10^{-5}
Fe				7.28×10^{-5}	5.74×10^{-5}	
Br			1.12×10^{-5}			
Pb					4.95×10^{-6}	

All samples show the presence of P, Ca, and Sr (fig: 1, 2, 3, 4, 5)

Samples No. (1), (2), and (5) shows the presence of Zn. Only sample No (2) shows presence of Br and Fe lead Pb appeared in sample No (4). Samples No 3 and 5 shows the presence of copper Cu. The appearance of Bromide and Lead is unexpected. The other elements may enter the human body from food sources. Sample No. (4) which belongs to a patient from Khartoum state lead may be pollutant. Bromine in sample No (2) which belongs to the patient from Atbara may be due to food contamination eg as bromated residue in bread. Lead, copper and zinc are micro nutrients which may be available in human food.

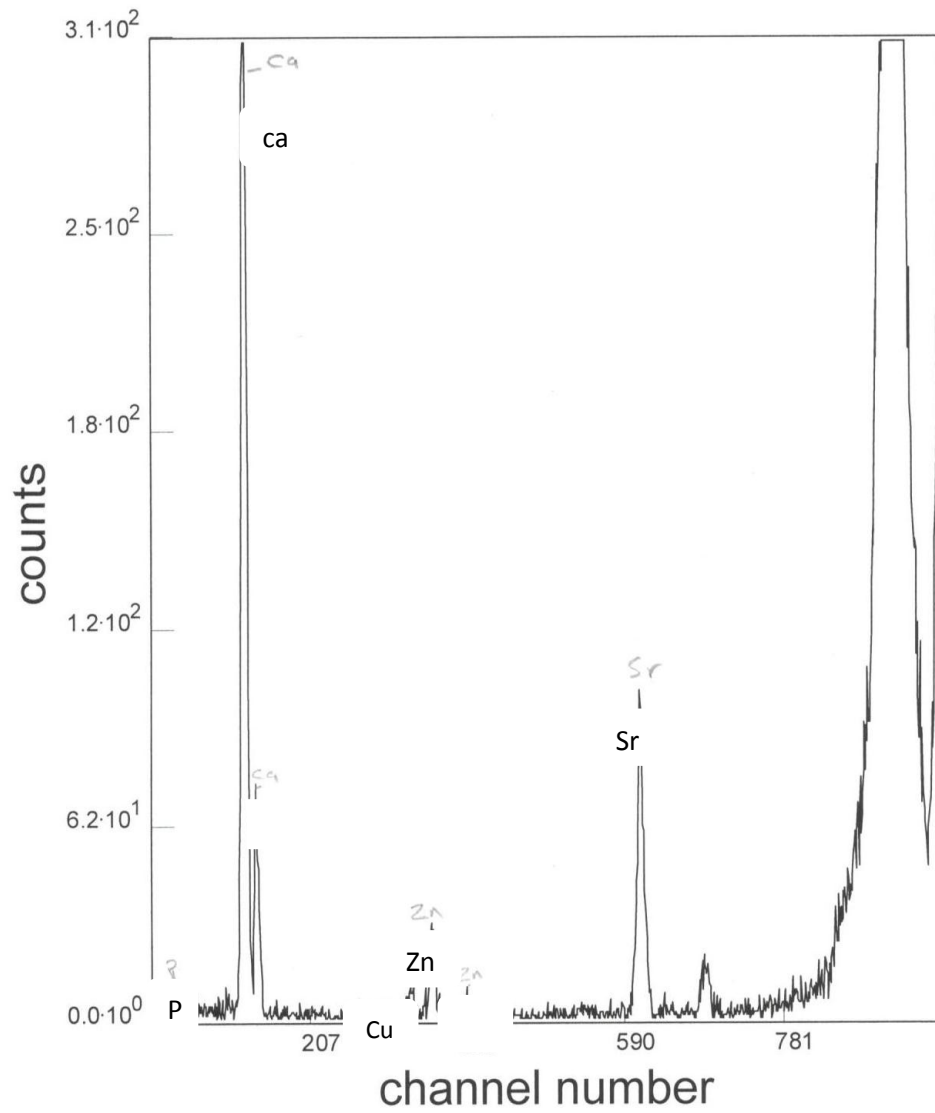


Figure (3-1): XRF scan of sample (1)

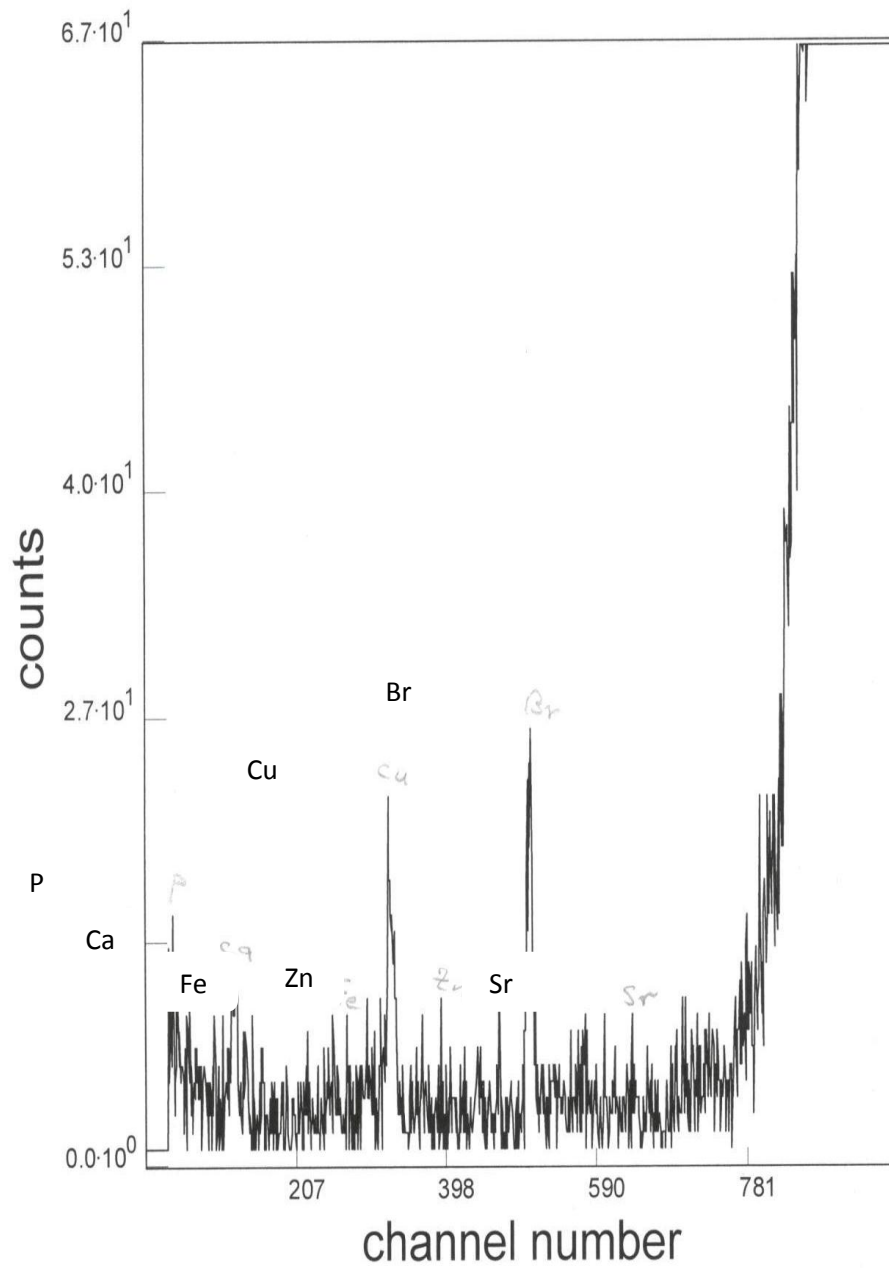


Figure (3-2): XRF scan of sample (2)

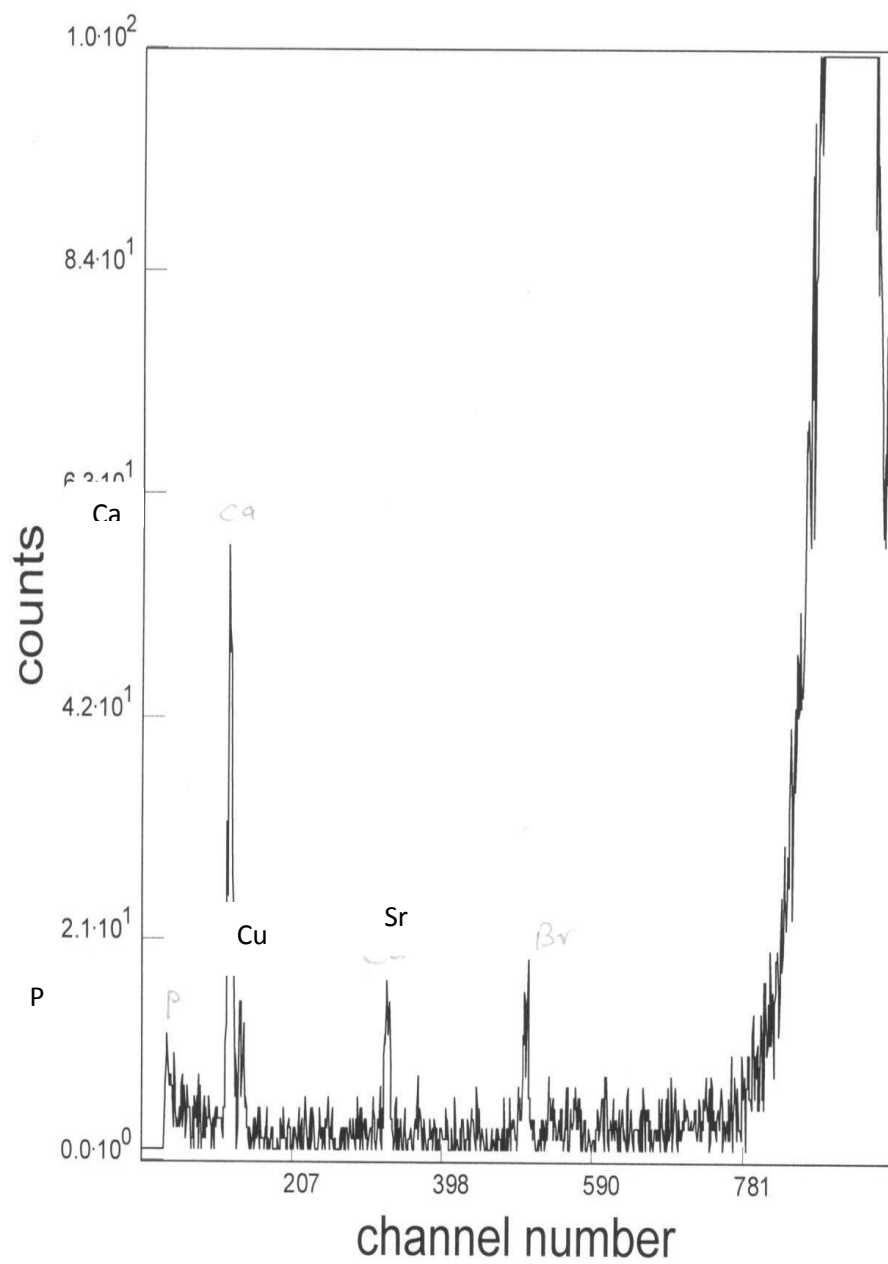


Figure (3-3): XRF scan of sample (3)

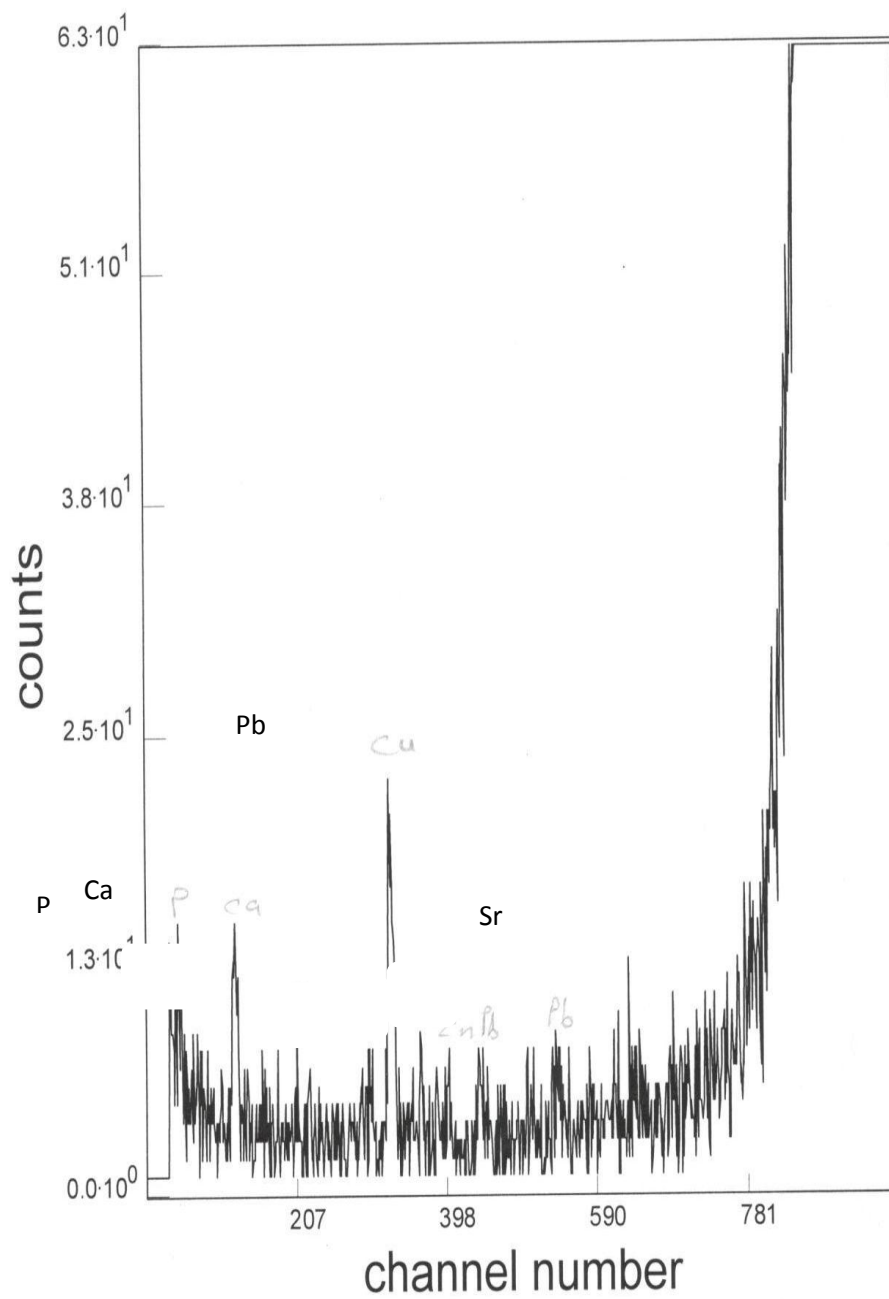


Figure (3-4): XRF scan of sample (4)

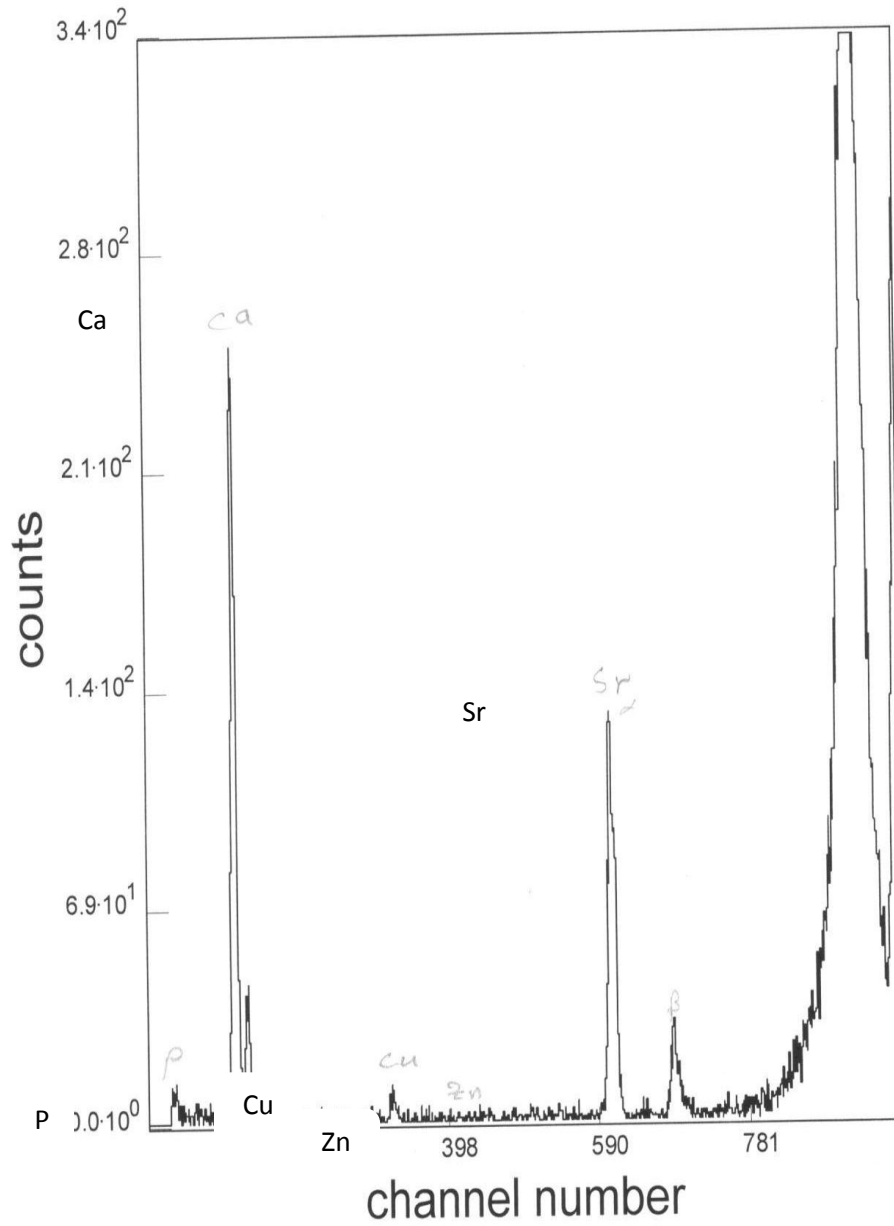


Figure (3-5): XRF scan of sample (5)

IR Results (fig 3.6) showed that the peak in region (3435.16 cm^{-1}) is due to (C H) stretching, the peak in region (1624.06 cm^{-1}) is due to (C O) stretching vibration, the peak in region (1319.43 cm^{-1}) is due to (O C) stretching, the peak in region (1041.42 cm^{-1}) is due to (N H) stretching, the peak in region (779.25 cm^{-1}) is due to (C C) symmetrical stretching.

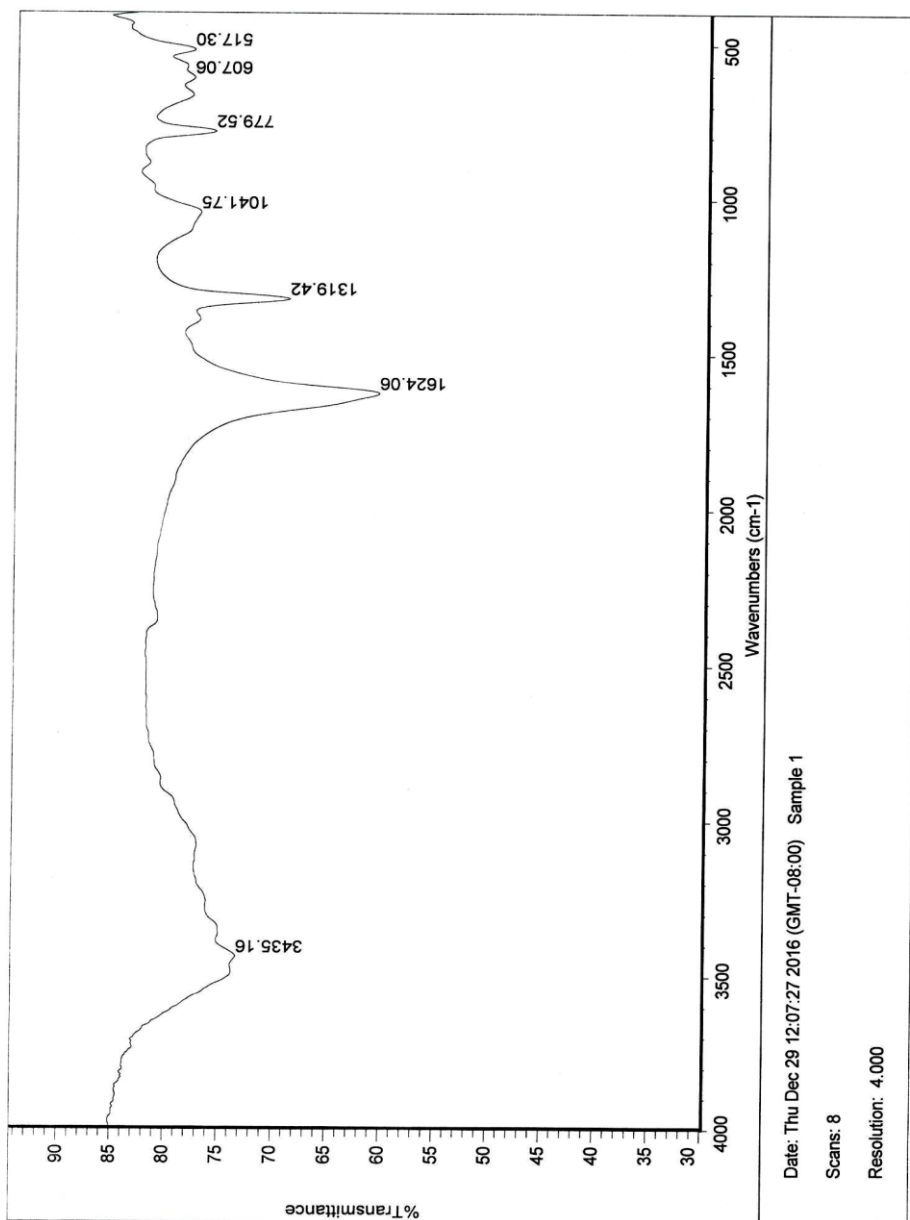


Figure (3.6): IR Spectrum of sample (1)

In figure (3.7) the peak in region (2937.76 cm^{-1}) is due to (C H) stretching, the peak in region (1460.99 cm^{-1}) is due to (P CH₂) bending, the peak in region (1634.03 cm^{-1}) is due to (C C) stretching, the peak in region (1375.47 cm^{-1}) is due to (P O) stretching, the peak in region (1055.36 cm^{-1}) is due to (R O) stretching, The medium band at (837.39 cm^{-1}) is due to (P O) stretching, the peak in region (957.50 cm^{-1}) is due to (C N) stretching, the peak in region (603.36 cm^{-1}) is due to bromine.

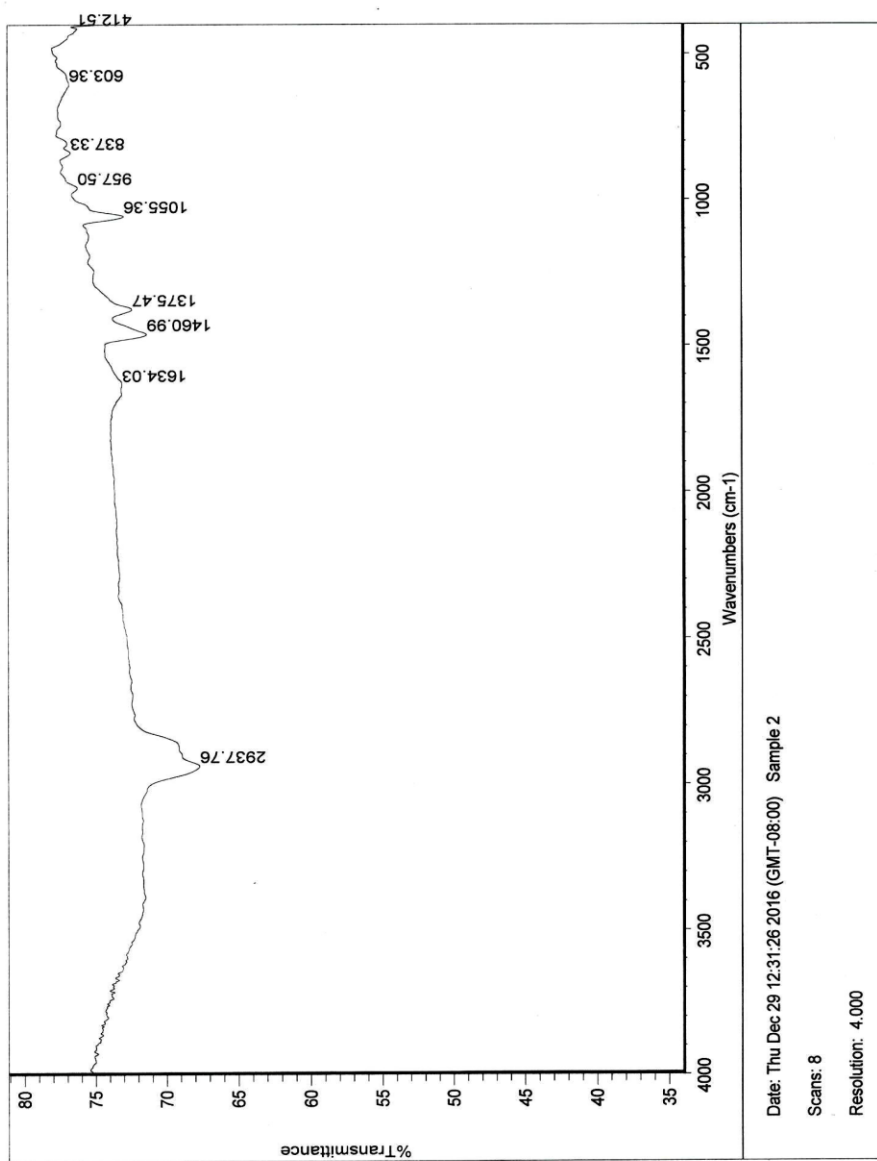


Figure (3.7): IR Spectrum of sample (2)

In Figure (3.8) broad peak at (3417.66 cm^{-1}) is due to (O – H)–stretching, the peak in region (1461.34 cm^{-1}) is due to (C – C) stretching, the peak in region (2939.86 cm^{-1}) is due to (C – N) stretching, the peak in region (1665.31 cm^{-1}) is due to (C = C) stretching, the peak in region (1054 cm^{-1}) is due to (N – H) stretching, the peak in region, ($736.11\text{ and }837.29\text{ cm}^{-1}$) are due to (C – N) stretching.

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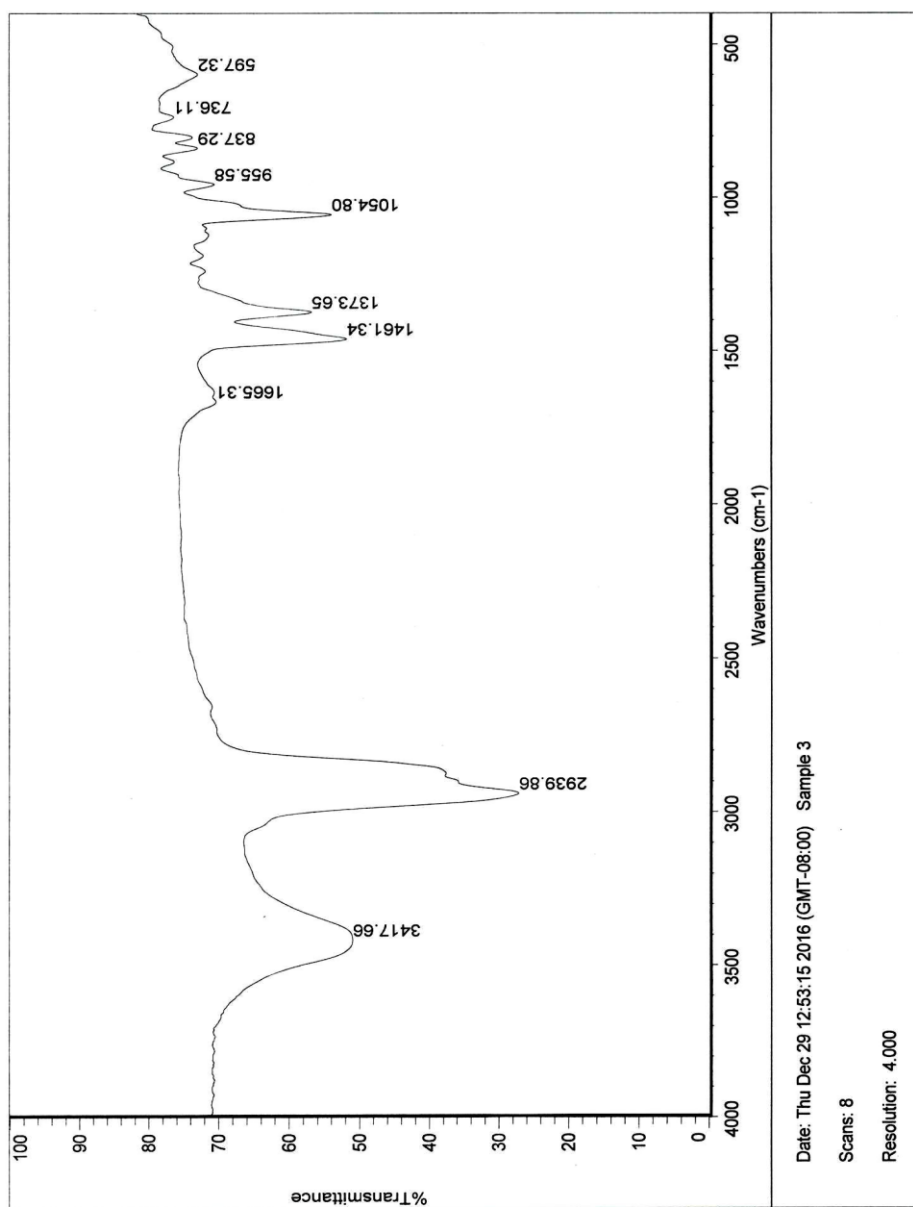


Figure (3.8): IR Spectrum of sample (3)

In Figure (3.9) the peak in region (1674.67 cm^{-1}) is due to (C=C) stretching, the peak in region (1119.49 cm^{-1}) is due to (C=C) bending, the peak in region (989.49 cm^{-1}) is due to (N-H) stretching, the peak in region (784.52 cm^{-1}) is due to (C-N) stretching of aromatic.

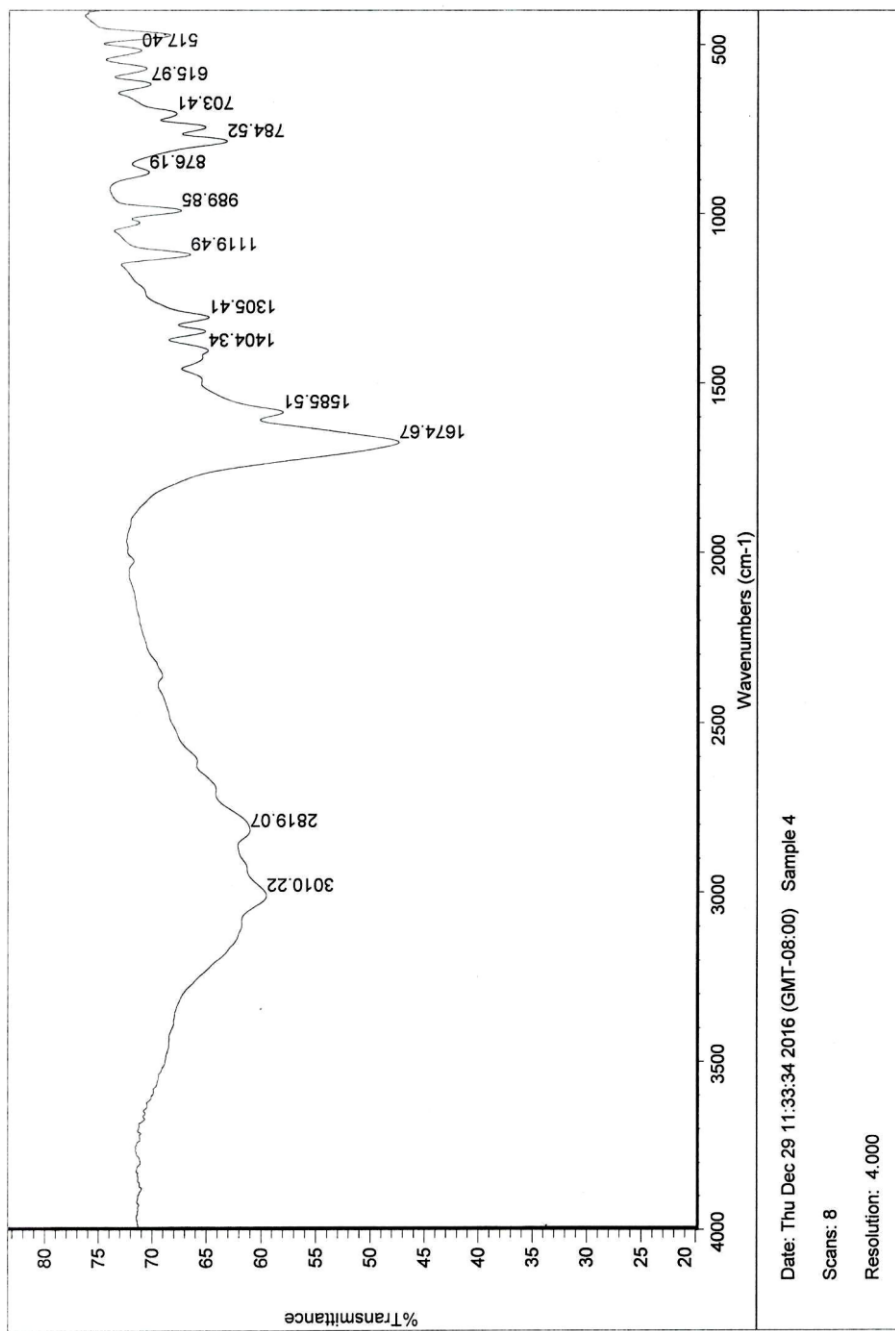


Figure (3.9): IR Spectrum of sample (4)

In figure (3.10) the broad peak in region (3429.26 cm^{-1}) is due to (O-H) stretching, the peak in region (1653.03 cm^{-1}) is due to (C=O) stretching, the peak in region (2920.55 cm^{-1}) is due to (C-H) stretching. the peak in region (1420.55 cm^{-1}) is due to (C-C) symmetrical bending, the peak in region (1039 cm^{-1}) is due to (P-O-C) aliphatic stretching.

Sample (1) and (4) are calcium oxalate, Sample (2) is calcium phosphate, and sample (3) is uric acid stone type.

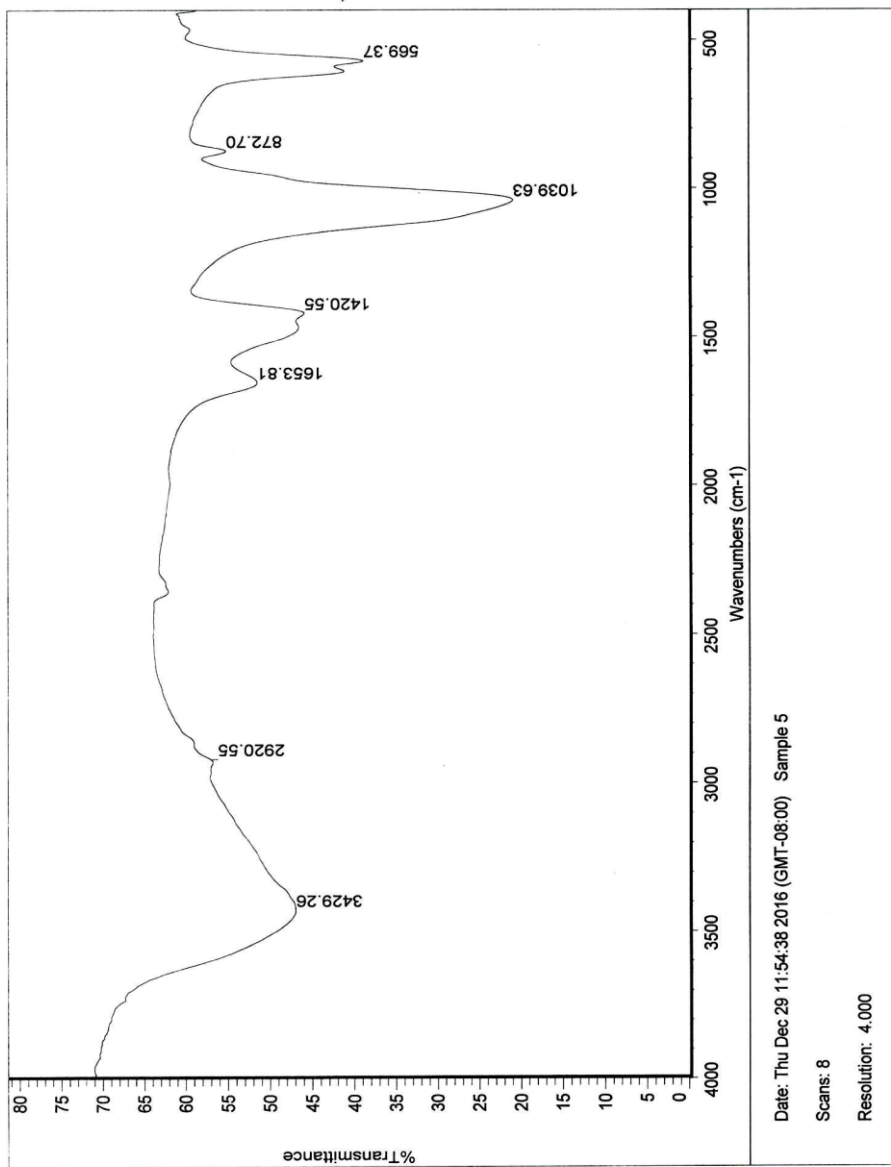


Figure (3.10): IR Spectrum of sample (5)

Conclusion

According to the obtained results we may conclude that the main type of kidney stones is calcium oxalate stones. The IR analysis showed the presence of functional groups indicating the occurrence of some phosphate and uric acid stones. We can also include that calcium as a metal ion is the main ion in the stone formation depending on the XRF results. Other elements compounds may also be considered as a part of kidney stone formation e.g.; Fe, P and Sr.

Phosphorous may be a part of phosphate stones. Iron oxides and hydroxides are insoluble in water and also most of strontium salts are insoluble, so they may also consider to be a part of the kidney stone constituents but in small scales.

Recommendations

- * The range of sampling may need to be widened to cover more number of patients with different ages, and to cover also different areas from the whole Sudan.
- * The sources of high calcium and high oxalate may need to be investigated eg; drinking water, vegetables and other types of food.
- * The types of kidney stones may also need to be detected, and also which part of the country is mainly affected by kidney stone diseases.
- * Also it may be important to study the relation between kidney stone diseases and renal failure.

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