بسم الله الرحمن الرحيم

Sudan University of Science and Technology

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Chemical Composition of Kidney and Gall stones of some Sudanese

التركيب الكيميائي لحصى الكلى والمرارة لبعض السودانيين

A thesis submitted in Fulfillment of the Requiredments for the Degree of Doctor of Philosophy in Chemistry

By

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إستهلال

قال تعالى :-

﴿وَإِذَا مَرِضْتُ فَهُوَ يَشْفِينِ﴾

صدق الله العظيم

سورة الشعراء – الآية (80).

Dedication

To my

Father and my Mather,

Husband,

Daughter

Brothers and sisters.

Acknowledgement

Praise to Allah Almighty, for giving me health and patience to complete this work.

I would like to express my deep sense gratitude to my Supervisor Dr Abd alsalam Abdallah Dafaalla for his guidance and encouraging.

Thanks to co-Supervisor Dr. Mohammed Suleiman for his active, Academic support, providing a wealth of information about the research subject. Deep thanks would go to the staff of the chemistry laboratories in Sudan University of Science and Technology,
It's a pleasure to thank all those who helped me in this search.

Abstract

Environmental, dietary habits, genetic, metabolic conditions, and life style factors play an important role in the formation of kidney and gall stones, which represent a prevalent and costly health problem. This study aimed to define patterns of stones and identify their chemical compositions in the collected samples using inductively coupled plasma optical emission (ICP), infrared spectroscopy (IR) and ultra violet spectrometer (UV) techniques. A total of 20 kidney and gall stone samples were collected randomly and surgically recovered from (11males and 9 females mean age 7-90 years) of Sudanese subjects. Analysis by inductively coupled plasma optical emission (ICP), was used to identify the elements composition of all collected kidney and gallstone samples. The results revealed that Ca, Na, K and Mg, found at high concentrations in contrasting with other elements among the collected samples. Also kidney and gallstone samples were analyzed by ultra violet (UV) technique and the obtained spectra showed that, all the samples absorbed energy at wavelength range205-230nm. This absorption attributed to presence of the carbonyl group which absorbs radiation at 220nm. In addition, the collected samples were characterized by infrared (IR) technique. The obtained IR spectra indicated that kidney stone samples (1, 3, 4, and 5) were composed from calcium oxalate and sample 2 was composed from Uric acid. Moreover, the IR results revealed that, all gall stone samples are composed of mixed stones. The study finding showed that kidney stones formation is common in male subjects, while gall stones formation is common in female subjects.

المستخلص

العوامل البئيه والعادات الغذائية والعوامل الوراثيه والظروف الايضية ونمط الحياة تلعب دورا هاما في تكوين حصاوات الكلي والمرارة والتي تمثل مشكلة صحية منتشرة ومكلفة .هدفت هذه الدراسة للتعرف علي انماط الحصاوي وتحديد تركيبتها الكيميائية لكل العينات باستخدام تقنية انبعاث البلازما البصرية المقترنة بالحث وتقنية الاشعة فوق البنفسجية والاشعه تحت الحمراء .جمعت 20 عينة من حصوات الكلي والمرارة عشوائيا وتم اخذها جراحيا من (11ذكور و 9 من الاناث) وكان متوسط العمر (7-90). تم تحليلها بواسطة تقنية انبعاث البلازما البصرية المقترنة بالحث(ICP) لتحديد مكونات العناصر في كل عينات الكلي والمرارة.كشفت النتائج ان الكالسيوم والصوديوم والبوتاسيوم والماغنيزيوم وجدت بتراكيز عالية علي عكس العناصر الاخري بين العينات .كما تم تحليل عينات حصوات الكلي والمرارة بتقنية الاشعة فوق البنفسجية واظهرت الاطياف ان جميع العينات تمتص الطاقة في نطاق طول موجي في المدىnm -205 230 ويعزي هذ الامتصاص لوجود مجموعة الكاربونيل التي تمتص الشعاع عند 220nm.بالاضافة الي ذالك فأن نتائج اطياف الاشعة تحت الحمراء دلت علي ان عينات الكلي (1,3,4,5)تتكون من اوكسالات الكالسيوم والعينة (2) كانت من حمض اليوريك . علاوة علي ذالك كشفت الاشعة تحت الحمراء ان جميع حصوات المرارة تتكون من أحجار مختلطة .اظهرت نتائج الدراسة ان تكوين حصوات الكلي شائعه عند الذكور بينما تكون حصوات المرارة شائعة عند الاناث.

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1-Introduction

1-1 Kidney stone

Kidney stones are a common disease in the general population, its prevalence being 5-10%, with a male predominance and an estimated recurrence rate of 50% (1). A Kidney stones, also known as renal calculi (singular calculus), are the most common disease of the urinary tract affecting about 10% of the global population (2) kidney stones are a common condition associated with significant morbidity because up to 12% of men and 5% of women will have Symptomatic kidney stone by the age of 70 years (3) .The male to female ratio is about 3:1 age and urinary pH have also been reported to be associated with the occurrence of common kidney stone(4). Kidney stones are small, hard deposits of mineral and acid salts on the inner surfaces of your kidneys. Normally, the substances that make up kidney stones are diluted in the urine. When urine is concentrated, though, minerals may crystallize, stick together and solidify. The result is a kidney stone. Most kidney stones contain calcium. (2). the size, shape, hardness and general appearance of the kidney stone also vary depending on the individual case. Although kidney stones are composed of many different compounds and are structurally unrelated, there is one common property among them (5). Behind the symptoms caused by the disease, the potential morbidity is high as demonstrated by the fact that from 10 to 15% of renal calculi require surgical treatment and between 20 and 30% to be hospitalized. (6) The majority of stone formers have disturbances either in the metabolism and excretion of stone constituents or in promoters and inhibitors of crystallization. Clinical and epidemiological studies have documented that several types of risk factors are involved in disease etiology, such as dietary habits, warm climate, and familial occurrence (7) .Renal stone for mars seem to eat more oxalate, more flesh protein and less vegetable fibers than their normal counterparts. Often their urine volume is low. These are environmental factors which govern nephrolithiasis. (8) The composition of urine from which these stones are formed and in which they grow is, in part, dependent on diet, including fluid intake. (9)

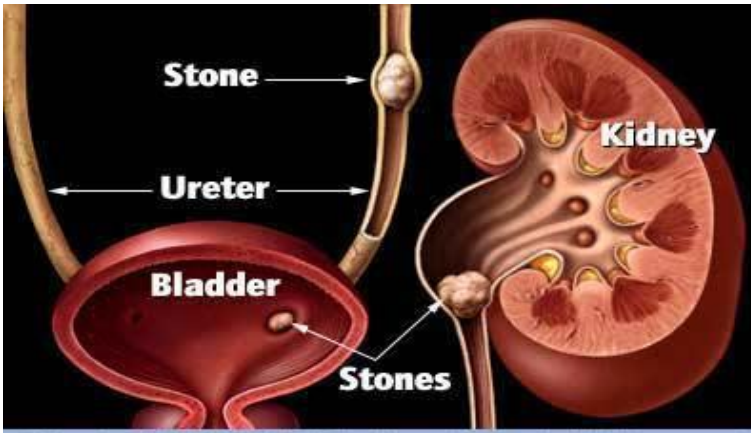


Figure 1.1 photographs of stones in kidney

1-1- 1 Classification of kidney stones

Urinary stones are typically classified by their location in the kidney, ureter, or bladder, or by their chemical composition. The chemical composition of kidney stone is quite varied, some are inorganic compounds such as calcium phosphate and some are organic salts such as calcium oxalate. Others are non-salt types of organic compound such as uric acid and cystine (5). Kidney stones come in different varieties, out of which four basic types more commonly found are Calcium-containing stones, Uric acid stones, Struvite, or infected stones and Cystine stones. (10)

1- Calcium stone

The majority about 75 percent of all urinary tract calculi are composed of calcium chemically mixed with oxalate (to form calcium oxalate stones) or phosphate (to form calcium phosphate stones). Of these two hard crystalline compounds, calcium oxalate stones appear most frequently, even though the two types may be mixed (11).

A-Calcium phosphate stones

Can be found in two forms, hydroxyl apatite [Ca_5 $(PO_4)_3OH$] and brushite($CaHPO_4H$ $_2O$) .Calcium hydroxyapatite is the component of the most common type of renal calculi.

B-Calcium oxalate

Which usually exists in the monohydrate (COM) or Dehydrate (COD) form? (2)Calcium stones are more common in men; the average age of onset is the third to fourth decade. Approximately 50% of people who form a single calcium

stone eventually form another within the next 10 years. The average rate of new stone formation in recurrent stone formers is about one stone every 2 or 3 years. Calcium stone disease is frequently familial (12)

2- Uric acid stone

About 10 percent of kidney stones contain uric acid, a waste product created by normal metabolism in the body (12). These form crystals in the urine, either alone or with other stone types. They are commonly due to an excessively high protein diet, obesity, or in patients who suffer with gout. Typically, these stones form in acidic urine (pH 5-6) and are not visible on plain x-ray. Uric acid stones are lower urine pH resulting from decreased ammonia excretion. It is thought that uric acid crystals can induce the Formation of calcium oxalate monohydrate stones by acting as a template for crystals to deposit and grow (13).

3- Cystine stones

These are rare stones occurring in 1% of stone patients, due to an inherited defect in amino acid transport within the kidney (13). Cystine stones occur because the kidneys do not reabsorb cystine properly. While cystine stones account for only one percent of all kidney calculi, they are difficult to treat and require life-long therapy (13).Cystine crystals appear in the urine as flat, hexagonal plates.

4- Struvite stone (Infection stone)

Between 10 and 15 percent of all kidney stones are associated with chronic urinary tract infections (11). Struvite stone (magnesium ammonium phosphate) are generally caused by a urinary infection with bacteria producing the enzyme (2). These stones can grow very rapidly to form a complete stone cast within the drainage system of the kidney. They are usually associated with urinary tract infections, which change the urinary environment to permit rapid stone growth. Consequently, the stone formed can become very large in size. If left untreated they can cause chronic infection, destroy the kidney, and may result in death. Struvite stones are often defined by their large size and stag horn edges (13)

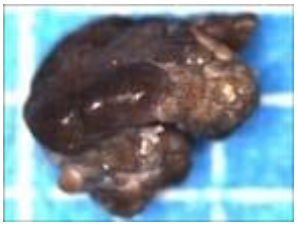
			
Calcium Oxalate Monohydrate deposited over Silica	Calcium Oxalate Monohydrate	Calcium Oxalate Monohydrate (coated with Triamterene)	Calcium Oxalate Monohydrate
			
Calcium oxalate Monohydrate	Calcium oxalate Dihydrate	Carbonate Apatite	Brushite

Figure 1.2 Kidney stone photographs

1.1.2 Risk factors for stone formation

There are no definite reasons for the formation of kidney stones. In some cases, urinary calculus may be predicted from the following risk factors; 24 hour urinary pH, amount of calcium (Ca), oxalate and uric acid excreted and level of urinary inhibitors, (14) in addition to the possible role of diet, Other diseases Cystinuria is a condition where lot of amino acid cystine is deposited which do not dissolve in the urine and thus lead to stone formation drinking water, in the last decade due to wrong food habits (15, 16).

1-Urinary Ph

In controls, the 24 hour urinary pH is higher than patients with urolithiais though the difference may be insignificant. Therefore, low urinary pH is considered as a risk factor particularly below the dissociation constant of uric acid. It is a major factor in uric acid lithiasis as because the urine is considered in a state of super saturation with uric acid in addition to being below the increase of the inhibition index for Ca oxalate crystal growth. Accordingly,

precipitation of uric acid becomes a function of pH rather than concentration. Therefore, in gouty diathesis (uric acid stone formation in primary gout), the 24 hour urinary pH is less than 5.5 and the uric acid content is within the normal range of 750 mg/day in women and 800 mg/ day in men. (17).

2- Hypercalciuria

Hypercalciuria is considered when the 24 hour urinary Ca content is above the upper limit of normal which is 300 mg in male's and250 mg in females (18); as the relative risk of stone formation increased with increasing urine Ca level and concentration (19,20) .Therefore, in patients with stone recurrence, strong enhancement of Ca Excretion,(21) and hypercalciuria is markedly more frequent than in first stone formers(22). It is reported that more than half of both men and women with recurrent stone formation have hypercalciuria. (23) In Saudi Arabia and Abu-Dhabi, hypercalciuria accounts for 9-29% of patients with Urolithiasis. However, a higher percentage than that and up to 81% of patients with urolithiasis is stated in current western reports. However, in controls, hypercalciuria is less commonly reported and at a lower relative frequency percent than in patients. Therefore, it seems that hypercalciuria is a risk factor in the presence of other urinary disturbances. as change in urinary pH, low urinary volume as a specific abnormality or hyperuricosuria. (17).

3- Hyperuricosuria

Hyperuricosuria is considered when the 24 hour also reported. More frequently, uric acid is evaluated in mg and hyperuricosuria then will be considered when the 24 hour urinary uric acid content exceeds 800 mg in men and 750 mg in women. tubular acidosis which is associated with hyper calciuria and ,hypocitraturia(24),and some genetic alterations, which are associated with hyperoxaluria (Hyperoxaluria is considered when the 24 hour urinary oxalate content exceeds the upper limit of normal which is 40 mg. Also an upper limit of normal higher or lower than that was considered), hyper cystinuria and hyper calciuria (25-27). In patients with urolithiasis, hyperuricosuria is the second metabolic urinary disturbance after hypercalciuria. Therefore, it is frequently reported in these patients. Meanwhile, in patients with uric acid lithiasis, uric acid concentration and 12 hour urinary excretion were found to be significantly greater in patients than controls. However, unlike Ca, there was no difference in the urinary uric acid excretion between first and recurrent stone formers. Alternatively, hyperuricosuria is one of the biochemical presentations which

differentiate between gouty diathesis (uric acid stones in primary gout) and hyperuricosuric Ca oxalate urolithiasis. (17)

4-Hyperoxaluria

Is considered when the 24 hour urinary oxalate content exceeds the upper limit of normal which is40 mg. Also an upper Limit of normal higher or lower than that was considered (28,29) Idiopathic Ca oxalate urolithiasis is a frequent and recurrent multi factorial disease. In patients with Ca oxalate stones (monohydrate or mixed mono and dihydrate), hyperoxaluria in 24 hour urine was the most common abnormality after hypocitraturia. (18). On the other hand, in controls, hyperoxaluria was not frequently identified and rarely reported. In Saudi Arabia, in patients with urolithiasis the mean 24-hour urinary oxalate content was higher in males than females and in either patients or controls it was higher than some current reports. Therefore, this risk factor seems to be prevalent in Saudi Arabia where an overall probability of stone formation is 20%.91. (17)

5- Some ionic correlations

a- Magnesium, calcium, potassium

In controls, in the 24 hour urine, magnesium (Mg) shows prominent direct correlation with uric acid, oxalate and phosphate. Meanwhile, Ca shows no correlation with Mg and an obvious direct correlation with uric acid thus leading to a low urinary Ca/Mg ratio in the controls. Furthermore, in the controls, the 24-hour urinary potassium (K) shows direct correlation with phosphate, uric acid and Ca but not oxalate. On the other hand, in patients with urolithiasis, the 24 hour urinary Ca shows prominent direct correlation with uric acid and oxalate. However, phosphate shows prominent correlation with Mg more than Ca Thus leading to an increase in the Ca/Mg ratio in these patients especially in those with hyperoxaluria hyperuricosuria, or both. In addition, the direct correlation between Ca and oxalate in the 24 hour urine of stone formers is well represented in the Ca stone composition. Meanwhile, it may be attributed to the presence of malon dialdehyde (MDA, one of the urinary lipid peroxides) in the urine of patients which show correlations with oxalate and Ca .Furthermore, in patients with urolithiasis, the 24 hour urinary K shows more correlations With phosphates than oxalate or uric acid and no correlation with Ca.

b- Uric acid, oxalate, phosphate

In controls, in the 24 hour urine, uric acid shows a direct correlation with phosphate and oxalate. However, its correlation with phosphate is far more than that with oxalate. This correlation is presented in the uric acid stone composition by the presence of variable amounts of oxalate (<40%) and only trace amount of phosphate. Meanwhile, in the 24 hour urine of stone formers, there is a mutual direct correlation between oxalate and phosphate. This correlation is well presented in the composition of phosphate stones by the presence of variable amount of oxalate (<40%). (17)

6- Correlations with family history of stones

Family history of stones is frequently reported in patients with urolithiasis (27, 28, 19, 29, and 30). Therefore, it is denoted that a family history of stones substantially increases the risk of stone formation in their siblings (19, 31) Furthermore, patients with urolithiasis, hypercalciuria and hyperuri cosuria Show prominent correlations with family history of stones (32, 33) Meanwhile, in the siblings of patients with Ca renal stones, hypercalciuria is considered as one of the reasonable predictors for those who are at risk of stone formation in both genders20. In addition, in patients with urolithiasis and family history of stones, the incidence of recurrence is higher than in those without a family history of stones28,35,36,. Therefore, family history of stones is considered as one of the items in the stone recurrence predictive score(36)

7- Wrong food habits

The large geographical variation in the incidence of renal stone disease was correlated with social and economic conditions(.34,37) In Europe, North America, Australia, Japan and Saudi Arabia, affluence has spread to all social classes and people have tendency to eat a large quantity of rich food (wrong food habits),)particularly the Saudi diet is over rich in protein and fat.(27, 38)Meanwhile, upper urinary tract stones are more frequent among affluent people with high animal protein consumption.(39,37)Furthermore, the risk of stone formation increased significantly with increasing body mass index among both men and women with urolithiasis. (39, 40, 41)Accordingly, the mean body mass index in patients with urolithiasis was significantly higher than that of controls. (27, 43, 41, 42)Although in Japan obesity is a risk factor for stone formation only in males, (42) in Saudi Arabia, it is a risk factor more common

in females than males.(27)Meanwhile, as the dietary and nutritional elements are important risk factors to the etiology of urinary calculi we will consider them as follows:(39)

a- Dietary animal protein

In patients with urolithiasis, the mean daily intake of dietary animal protein was significantly higher than that in controls.(27,28 , 43) Mostly the high intake of dietary animal protein was directly associated with the risk of stone formation.(39,37,44,45)

c- Dietary fat

In patients with urolithiasis, the mean daily intake of fat was significantly higher than that in controls. (27, 46) Meanwhile, in patients, it is reported that the daily intake of fat was higher in men than women and the difference was statistically highly significant. (45).

c- Energy

In patients with urolithiasis, the mean of total daily intake of energy was commonly significantly higher,(27, , 46) less commonly lower than that of controls. (27, 43) Furthermore, in young women, sucrose intake showed relative risk of stone formation.(47, 48) In accordance with this, in animals, the deposition of Ca oxalate in the kidney was the greatest with sucrose, fructose, orbital and the least with glucose.(48)

d- Dairy products and calcium supplements

The dietary Ca intake is inversely associated with the risk of kidney stones (.31, 49, 45, 47, 50-52) Meanwhile, there is no evidence of any rise in the risk of stone formation in relation to dietary Ca intake (52) Accordingly, in patients with urolithiasis, the mean dietary Ca intake was commonly lower, (17,27, 53, 46) less commonly significantly higher than that of controls. (54) However, the intake of supplemental Ca was positively associated with risk of stone formation in women when consumed without meals. (47)

e- Magnesium and potassium

In patients with urolithiasis and hyper absorptive hypercalciuria, oral supplementation of Mg is favorable as it decreases Ca absorption and increases

Mg absorption which as an inhibitor reduces risk factors of the disease131. However, K intake was found to be inversely related to the risk of stone formation. (44)

f- Vitamins

It was found that, in both men and women, there are no correlations between the risk of stone formation and the intake vitamins B6 or C even when taken in large doses (55, 56).

8- Drinking water

The possible correlation between the drinking water and prevalence of urolithiasis was considerably investigated. There is no evidence of any rise in the risk of stone formation in relation to tap water hardness (27, 39) and underground water in the Western Region of Saudi Arabia 28. Meanwhile, drinking of soft water over rich alone or with an additional factor was associated with high prevalence of urolithiasis (57-59) However, it was reported that in patients with urolithiasis, drinking soft water was not associated with any changes in the urinary parameters110. In addition, whether in patients with urolithiasis or normal subjects 61,62,63 drinking bicarbonate alkaline water with a high content of Ca (370-380 mg/L) leads to an increase in the urinary Ca by 50% in patients 98and approximately 80 mg/day in normal subjects 64. Furthermore, in patients with urolithiasis, drinking mineral water with high sulphate and intermediate Ca content (123.9mg/l)65 or the replacement of one liter of the usual fluid intake with mineral water in normal subjects108, elevated the urinary Ca as well.. There is a possible correlation between the drinking water types and prevalence of urolithiasis. Therefore, whether in patients with urolithiasis or normal subjects, drinking hard water should be avoided due to its effects on the urinary risk factors (64,63, 62). Mean while, in patients ,for increasing urinary volume to prevent stone recurrence the use of low Ca content water or tap water is recommended (63,62).

1.1.3 Symptoms of kidney stones

Kidney stones typically leave the body by passage in the urine stream, and many stones are formed and passed without causing symptoms. If stones grow to sufficient size (usually at least 3 millimeters, they can cause obstruction of the ureter. Most the kidney stones do present themselves with many symptoms. However, if the size of the stone is large enough, then it could lead to blockage in the urinary tract resulting in severe pain while passing urine. In such condition, the person may feel pain in the lower abdomen. He may feel nausea accompanied with vomiting. Sometimes Blood may be seen in the urine (bleeding) this is because the stone will irritate the ureter and cause blood. However all the cases with blood in urine do not really indicate the presence of Kidney stones? Some other reasons may also cause blood spots in urine. Frequent and painful urination may also be a symptom of the presence of kidney stone. Many people feel stinging or burning sensations while passing urine. Tenderness in kidney, abdomen and Urinary tract infection may also be signs of kidney stones. (64)

Common symptoms of kidney stone: Hematuria, Severe colicky flank pain, Nausea/vomiting, severe colicky back pain and Hypertension (65).

1.1.4 Treatments for kidney stones

1- Water and Juices

It is a major factor in uric acid lithiasis as because the urine is considered in a state of super saturation with uric acid (64)

2- Drug treatment

Thiazide lowers urine calcium resulting in a fall in calcium oxalate and calcium phosphate super saturation.

3- Allopurinol

Allopurinol blocks uric acid production, reducing heterogeneous nucleation of calcium oxalate by both uric acid and monosodium urate.

4- Potassium citrate

Potassium citrate reduces urinary saturation of calcium salts by completing calcium and reducing ionic calcium concentration(66).

5- Surgery

Surgery is one of the common methods used to remove the stone. Surgery is usually needed if the stone is too large to pass on its own, or the stone is growing, or the stone is blocking urine flow and causing an infection or kidney damage. Laparoscopy is one of the methods of minimally invasive surgery (MIS) that can be useful in different surgery operations (67,68) This method of surgery has a lot of advantages in comparison with the traditional open surgery method such as less pain, less infection, less time of recovery (69). In contrast with these valuable advantages, a significant disadvantage of this method is missing a considerable amount of surgeon's tactile sensing that is exploited to recognize different organs and tissues (70,71).

6- Extracorporeal shock-wave lithotripsy

Extracorporeal shock-wave lithotripsy is used to remove stone slightly smaller than a half an in inch that are locate near the kidney. This method uses ultrasonic wave or shock waves to break up stones. Then, the stones leave the body in the urine.

1.1.5 Inhibitors of stone formation

Urine contains potent inhibitors of nucleation, growth, and aggregation for calcium oxalate and calcium phosphate but not for uric acid, cystine, or struvite. Inorganic pyrophosphate is a potent inhibitor that appears to affect calcium phosphate more than calcium oxalate crystals. Citrate inhibits crystal growth and nucleation, though most of the stone inhibitory activity of citrate is due to lowering urine super saturation via complications of calcium. Other urine components such as glycol proteins inhibit all three processes of calcium oxalate stone formation. As a consequence of the presence of these inhibitors, crystal growth in urine is slow compared with growth in simple salt solutions, and the upper limit of Meta stability is higher (72)

1.1.6 Stone passage

Non obstructing stones produce no symptoms or signs apart from hematuria. Stone passage produces renal colic that usually begins as a mild discomfort and progresses to a plateau of extreme severity over 30–60 minutes. If the stone obstructs the uretero-pelvic junction, pain localizes to the flank; as the stone moves down the ureter, pain moves downward and anterior. Stones at the uretero-vesicular junction often cause dysuria and urinary frequency mistaken for infection. Colic is independent of body position or motion and is described as a boring or burning sensation associated with nausea and vomiting. Stones less than 5 mm in diameter have a high chance of passage; those of 5–7 mm have a modest chance (50%) of passage, and those greater than 7 mm almost always require urological intervention (73)

1.1.7 Pathogenesis

Urinary stones usually arise because of the breakdown of a delicate balance. The kidneys must conserve water, but they must excrete materials that have a low solubility. These two opposing requirements must be balanced during adaptation to diet, climate, and activity. The problem is mitigated to some extent by the fact that urine contains substances that inhibit crystallization of calcium salts and others that bind calcium in soluble complexes. These protective mechanisms are less than perfect. When the urine becomes supersaturated with insoluble materials, because excretion rates are excessive and/or because water conservation is extreme, crystals form and may grow and aggregate to form a stone (72)

1.2 Gall stones

Gallstones are abnormal stone masses formed in the gallbladder or the intra hepatic bile ducts and infrequently also migrate to the common bile duct or the intestines (74, 75). Gallstones are frequently composed of more than one crystalline compound, although the exact mechanism of formation of multi component stones is not clearly understood (76) The presence of gall-stones in humans has been identified in the mummy of an Egyptian priestess dated back to about 1500 BCE (77) Gallstones formed when there are much cholesterol and other substances in the bile which form crystals and changed to hard stones in the gallbladder. Gallstones also formed when the gallbladder does not empty properly (78, 79).

Gallstones are more prevalent in people who are obese because increasing body mass is associated with an increased production of cholesterol by the liver (80).

Gall bladder stone disease is one of the major surgical problems in the Sudanese population and it accounts for many hospital admissions and surgical inter Vent ions. (81), is still a major health problem all over the world. 20–30% of western and around 10% of non-western population have been affected by gallstones [82]. Ultrasound studies indicate mean prevalence rates of 10–15% in adult European, and of3–5% in African and Asian populations [83]. More than 80% of gallstone carriers are unaware of their gallbladder disease [84, 85], but about 1–2% per year of patients develop complications and need surgery [86]. In the US, gallstone disease has the most common inpatient diagnosis among gas trointestinal and liver diseases [87]. The prevalence among adults is approximately 10-15% for men and 20% for women in Europe and North America. Age, gender and ethnicity are the most important factors affecting prevalence (88).

1.2.1 Classification of galls stones

Gallstones formation great variations in appearance, structure and chemical composition of the different types of stones were clear proof of different modes of origin (89). They are generally Classified into three major types, Pure cholesterol, Pure Pigment and mixed. (90). which can form in the gallbladder or within the bile ducts of the liver (91). In the Western world, the major constituent of gallstones is cholesterol, which comprises 50-98 % of the dried substance stone(92). Other constitutes may include fatty acids, triglycerides, proteins ,polysaccharides, as well as calcium bilirubinate, calcium carbonate and calcium bicarbonate (93)

Cholesterol gallstones are higher prevalence than pigment gallstones. Chemical classification and location of biliary calculi differ in various parts of the world and change over time because of nutritional, socio-economic and demographic constituents of different types of gallstones [94) Diet affected the type of gallstone formation. Studies reported that Consumption of fried foods and animal lipid increased risk of cholesterol gallstone formation, while intake of carbohydrate increases risk of pigment gallstones formation. Suggesting diet influenced the type of gallstone formation [, 95, and 96]. In the Western world, approximately 70% of gall stone carriers exhibit cholesterol gallbladder stones and 30%exhibit black pigment gallbladder stones. In East Asia, there is a high

prevalence of brown pigment stones residing in the bile ducts, and causing potentially devastating cholangitis. Nevertheless, also in these countries, prevalence of cholesterol gall stones increases, supposedly caused by the introduction of Western diet. Because of increased prevalence of overweight or a higher proportion of elderly subjects in the population, the prevalence of gallstone disease may further increase in the near future (97).

1-Pure cholesterol stones

Cholesterol stones form due to an imbalance in the production of cholesterol or the secretion of bile.92 Cholesterol gallstones have been estimated to account for 75-90% of gallstones prevalence in Western countries (98). Cholesterol stones, or mixed stones with cholesterol as the main component, were found in 60% of patients in a recent study (98).

2-Pigment stones

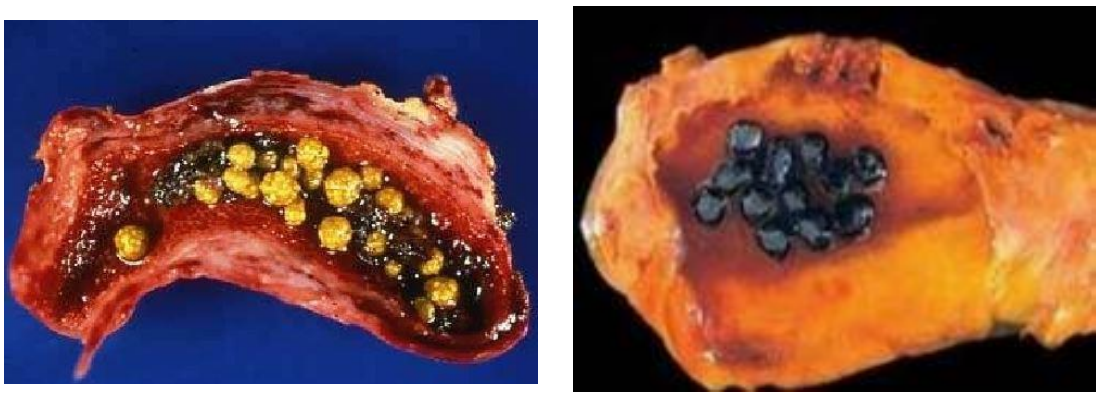
Pigmented stones are primarily composed of bilirubin, which is a chemical produced as a result of the normal breakdown of red blood cells (92) . And can further be divided into black and brown stones (99)

a- Black pigment stones

Black pigment stones are primarily composed of calcium bilirubinate. Other Important components are calcium carbonate and calcium phosphate in polymerlike Complexes with musing glycolproteins. In case of hemolysis, biliary excretion of bilirubin may increase 10-fold with increased risk of calcium bilirubinate precipitation (99).Black pigment stones may be associated with physiological conditions including hemolysis and increased production of un conjugated bilirubin (101) or of pre-hepatic origin like spherocytosis, sickle cell disease, thalassemia, and malaria (99). Higher prevalence of black pigment than cholesterol gallstones are found in developing countries and in Asian populations (102).

b- Brown pigment gallstones

In contrast to black pigment stones, their brown pigment counterparts are formed in the bile ducts. They are primarily composed of calcium salts of UN conjugated bilirubin and varying amounts of cholesterol and protein. Brown pigment stones are associated with chronic bacterial infection of the bile ducts by Escherichia coli, Bactericides', and Clostridium, and parasites.(100) .Brown pigment stones are completely different from other stones because they are caused by bile stasis and infection; namely by Escherichia coli, which produces enzymes, such as beta glucuronidase and phosphor lipases (103) The major chemical constituent of brown stones is calcium palmitate (up to 25%), whereas calcium phosphate and calcium carbonate are both usually found in black stones. (104).



Pigment stones pure cholesterol

Figure 2.2 Gall stone photographs

1.2.2 Risk factors for stone formation

The most common risk factors for gallstones formations are old age, female gender, obesity and diabetes mellitus. In addition, the risk disease occurrence increases with getting older in both genders and a more significant prevalence among females than males Data suggest that physical activity, modifying life style, decreasing weight gain and avoiding spicy and fried foods would result in prevention of gallstones prevalence (105)

1-Age

All epidemiological studies showed that increasing age was associated with an increased prevalence of gallstones. Gallstones are four to 10 times more

frequent in older than younger subjects. Biliary cholesterol saturation increases with age biliary cholesterol saturation increases with age, due to a decline in the activity of cholesterol 7α hydroxylase, the rate limiting enzyme for bile acid synthesis. Age appears to have an effect on the incidence of gallstone disease. Gallstone disease before 20 years of age is a rare occurrence. In infants and children, the most common stones are pigment stones, which are related to hemolysis or chronic diseases. Typically, only 0.15% to 0.22% of children will have gallstones, and children account for less than 5% of all cholecystectomies. The increased incidence of gallstones with age is seen across all ethnic groups. A study in Taiwan confirmed that increasing age had a direct relationship with the development of gall stones simply due to the long-term exposure to other risk factors irrespective of locality or standard of living.106

2-Gender

Gender is one of the most powerful influences on gallstones, which are more common in females during their fertile years as in males.(107)The higher prevalence of cholelithiasis among females is most likely related to estrogen increasing biliary secretion of cholesterol and progesterone reducing bile acid secretion by increasing gallbladder stasis(108) The risk of gallstone formation in females is increased by taking oral contraceptives.(109) The influence of the female sex hormones has been studied in normal females, during pregnancy, and in women using oral contraceptives. The risk of gallstones is greater in younger females (109).Pregnancy favours gallstone formation through the hormonal influences on bile composition (increased biliary cholesterol secretion, decreased and unbalanced bile acid pool). (111)

3-Obesity

Gallstones are more prevalent in people who are obese because increasing body mass is associated with an increased production of cholesterol by the liver. Periods of rapid weight loss are also associated with gallstone formation and people are more likely to become symptomatic during this time. This is possibly due to an increase in the relative amount of cholesterol in the gallbladder and reduced gallbladder contractility.(112) Obesity is an important risk factor for gallstone disease, more so for women than for men. Epidemiological studies have found that the lithogenie risk of obesity is strongest in young women and that slimness protects against cholelithiasis.(107)

1.2.3 Symptoms of gall stones

The most common symptoms include abdominal pain after eating a fatty meal, Pain in the abdomen and back (113). This disease doesn't cause any symptoms in most patients Many times gallstones are found by chance on an abdominal x-ray or ultrasound done for other reasons. Unless symptoms of pain, nausea, vomiting or fever are present, no additional testing or intervention may be needed. Symptoms arise when a gallstone blocks the flow of bile out of the gallbladder or through the bile ducts. A gallstone in the common bile duct is called choledocholithiasis and may cause intermittent or constant discomfort. The pain of choledocholithiasiss is usually localized in the upper abdomen, and can radiate in the right shoulder, may last many minutes to hours, and be associated with sweating, nausea, vomiting (91).

1.2.4 Treatments for gall stones

Treatment depends on the size and location of the gallstones, but may include:

a- Medications treatment

Some drugs can dissolve gallstones but this treatment is only rarely given, due to side effects and variable success rats [114]. Treatment of disease involves narcotic pain relievers or antispasmodic agent or pain control with none steroidal anti- inflammatory drugs. However, comparison studies have shown that NSAIDs (none steroidal anti- inflammatory drugs) provide faster and more effective pain relief. The patient should fast as part of the conservative management of biliary colic and to avoid the release of endogenous Cholecystokinin.

b- Surgical treatment

Cholecystectomy, usually laparoscopic, is recommended for most patients with symptomatic gallstones to remove the stones from the bile duct or the entire gallbladder [115,116]. Lithotripsy is a machine shatters stones by sound waves and used for people with small stones [117].

d- Dietary treatment

Limiting or eliminating fatty foods and dairy products is recommended. Previous studies reported that risk of gallstone was positively associated with

intake of meat, energy, fat and saturated fat, but negatively associated with intake of vegetable and fiber. Dietary calcium decreases cholesterol saturation of gallbladder bile by preventing the re absorption of secondary bile acids in the colon.Coffee components decrease cholesterol crystallization in bile due to stimulation cholecystokinin release. Coffee consumption inversely correlated with gallstone prevalence. [117-123].

1.2.5 Pathogenesis

Cholesterol gallstone formation can be arbitrarily divided into 3 stages: cholesterol saturation, nucleation, and stone growth Cholesterol solubilization and Super Saturation .Cholesterol is insoluble in aqueous solution and requires some vehicle to render it soluble in bile.(124). This is overcome by secretion of phospholipids and bile salts along with cholesterol. The biliary lipids (lecithin, cholesterol, and bile salts) are secreted into the hepatic canaliculi by adenosine tri-phosphate dependent transport proteins (125)

Cholesterol and lecithin combine to form Meta stable UNilamellar vesicles, while the bile salts, after reaching a critical concentration, form simple micelles . The dynamic interaction of unilamellar vesicles with the simple micelles leads to the formation of mixed micelles during the passage through the biliary tract into the gallbladder.(126, 127)

1.2.6 Nucleation of cholesterol crystals

Nucleation is the process by which cholesterol monohydrate crystals form and agglomerate .7 when the vesicles and mixed micelles interact in the gallbladder, the micelles remove phospholipids from the vesicles in preference to cholesterol, thus making vesicles rich in cholesterol. The remaining vesicles are relatively enriched in cholesterol and prone to nucleation. After nucleation, crystallization of the cholesterol can then occur eventually leading to the formation of gallstones (126).

1.2.6 Cholesterol stone growth

The nucleated cholesterol monohydrate crystal serves as the nidus for the growth of cholesterol stones (126). Repeated deposition of cholesterol on the nidus leads to stone enlargement. Growth of stones is most likely a discontinuous process that is punctuated by deposition of rings of calcium bilirubinate and calcium carbonate as the daily inter-play of cholesterol, bile

salts and phospholipids continue (126). The pathogenesis of pigment gall stones due to a lack of epidemiological studies. There are two types of pigment gall stones: black and brown gallstones. Black pigment stones are composed of an insoluble bilirubin pigment polymer mixed with calcium phosphate and carbonate.(13)

Precipitation of calcium bilirubinate occurs whenever the ionic product of calcium and UN conjugated bilirubin exceeds its solubility product. The major factors causing black pigment stones are hemolytic anemia's, ineffective erythropoiesis, and increased production of bilirubins (caused by hereditary spherocytosis, thalassaemia, sickle cell disease, liver cirrhosis, malaria, and ineffective erythropoiesis).(128) .Most of the brown pigment stones are formed in the bile ducts as a consequence of some infection and stasis of bile flow.13 Chemically they are calcium salts of long chain fatty acids and cholesterol. (128).

1.3 previous studies

There are many studies in this area carried by

Due to the increase in the number of urinary calculi disease cases in Jordan, stone samples were collected from patients from various Jordanian hospitals. This study concentrates on the effect of trace metals in patients of urinary calculi (132). 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 (CaOax), 29 were mixed calcium oxalate/ uricacid, 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 acid urate/sodium acid urate) and three were pure cystine calculi. The concentration measurement of Ca and other trace 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 concentration of most of the trace metals that were analyzed was (Ca = 48.18, Na = 1.56, K = 0.9, Mg = 3.08, 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 higher than 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. P and S ions are present in few calculi stones as traces (132).

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 (UA); 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 (133).

Inductively Coupled Plasma- Atomic Emission Spectroscopy(ICP-AES) was used to analyze human gall stones in Ag, Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga , K, La, Mg, Mn, Mo, Hg, Na, Ni, P, Pb, Sb, Sc, Sr, Th, Ti, U, V, W, and Zn129(.Thirty gallstones were collected during surgery from patients with cholesterol, pigment and mixed stones. The results of quantitative elemental analysis revealed that, out of 34 elements, 18 elements Ba,Be, Bi, B, Cd, Co, Ga, La, Mn, Mo, Ni, Sc, Th, Ti, Ti, U, V, and W were not observed in any of the stones. While the concentration of other elements were in the following order>Ca>Cu>Mn>Sr>Zn>Na>Mg>Pb>Fe ≈ Al≈ K ≈Hg>Sb>As ≈ Ag ≈ Cr. Trace metal concentration in patients from Erbil governorate gallstone were Compare with that obtained from patients from other countries(134).

Fourier transform infrared spectroscopy (FTIR) has been carried out to analyze the organic and inorganic constituent in human gallstones (n = 34) from Rajah Muthiah Medical College and Hospital. Statistical correlations between stone weight, age, stone type, number of pregnancy, food habit and chemical composition of gallstones were performed (135). The quantitative estimation was calculated for the particular peaks of bilirubinate salt (3421 cm–1), cholesterol (2932 cm–1), calcium palmitate (2849 cm–1) and calcium carbonate (1448 cm–1). The study reveals that mixed and brown type gallstones were

predominant whereas black stones were less frequent in the selected region. Calcium carbonate is found in all the stones, and it is in a higher amount in mixed stones. The present study shows that no pure cholesterol stone was found in the selected patients and mixed gallstones are high in the selected area, brown stones being seldom found. Mixed varieties of gallstones contained more cholesterol, calcium carbonates, calcium palmitate and fewer amounts of bilirubin compounds. Black pigmented stones contained a lesser amount of cholesterol, calcium carbonate, calcium palmitate and bilirubinate compounds whereas brown stones contained a higher amount of bilirubinate and mixed stones contained an intermediate amount of these four compounds (135)

The aim of the study

The aim of this study is to analyze qualitatively and quantitatively kidney and gall stones collected from twenty Sudanese patient using inductively coupled plasma optical emission, infrared spectroscopy and ultra violet spectrometer techniques to know the components of these stones and the quantities of these components .

2-Materials and Methods

2.1 Materials

The stones sample were surgically recovered from 20 (11 males and9 female, mean age 7 –90 years) patients in Sudan. The table below show the age of subjects and localization of stones.

Table 2: 1 Samples collections

Samples	Age	Gender	Localization Of Stones
Sample 1	82	Male	Kidney
Sample2	70	Male	Kidney
Sample3	40	Male	Kidney
Sample4	55	Male	Kidney
Sample5	90	Male	Kidney
Sample6	65	Male	Kidney
Sample7	60	Male	Kidney
Sample8	58	Male	Kidney
Sample9	7	Female	kidney
Sample10	55	Female	Kidney
Sample11	45	Male	Gall
Sample12	40	Female	Gall
Sample13	54	Female	Gall
Sample14	26	Female	Gall
Sample15	68	Male	Gall
Sample16	53	Male	Gall
Sample17	45	Female	Gall
Sample18	44	Female	Gall
Sample19	20	Female	Gall
Sample120	50	Female	Gall

2.2 Chemicals

- Hydrochloric acid (36-38%) LOBA chemie India
- Nitric acid (96-72%) LOBA chemie India
- Anhydrous potassium bromide LOBA chemie India

2.3 Instruments

2.3.1 Inductively coupled plasma optical emission spectroscopy (ICP/OES)

ICP/OES is one of the most powerful and popular analytical tools for the determination of trace elements in a myriad of sample types. The technique is based upon the spontaneous emission of photons from atoms and ions that have been excited in a RF discharge. Liquid and gas samples may be injected directly into the instrument, while solid samples require extraction or acid digestion so that the analyses will be present in a solution. The sample solution is converted to an aerosol and directed into the central channel of the plasma. At its core the inductively coupled plasma (ICP) sustains a temperature of approximately 10 000 K, so the aerosol is quickly vaporized. Analyze elements are liberated as free atoms in the gaseous state. Further collision excitation within the plasma imparts additional energy to the atoms, promoting them to excited states. Sufficient energy is often available to convert the atoms to ions and subsequently promote the ions to excited states. Both the atomic and ionic excited state species may then relax to the ground state via the emission of a photon. These photons have characteristic energies that are determined by the quantized energy level structure for the atoms or ions. Thus the wavelength of the photons can be used to identify the elements from which they originated. The total number of photons is directly proportional to the concentration of the originating element in the sample (129)

2.3. 2. Ultra violets spectrometer

The UV spectra were obtained for dilute solution (1 mg in 100 ml of solvent). A portion of this solution was transferred to a silica cell. A matched cell containing pure solvent was prepared, and each cell was 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 (130)

2.3.3Fourier transforms infrared spectroscopy

Infrared spectroscopy is certainly one of the most important analytical techniques available to today's scientists. One of the great advantages of infrared spectroscopy is that virtually any sample in virtually any state may be studied. Liquids, solutions, pastes, powders, films, fibers, gases and surfaces can all be examined with a judicious choice of sampling technique. This type of instrument employs an interferometer and exploits the well established mathematical process of Fourier-transformation. Fourier-transform infrared (FTIR) spectroscopy has dramatically improved the quality of infrared spectra and minimized the time required to obtain data. In addition, with constant improvements to computers, infrared spectroscopy has made further great strides. Infrared spectroscopy is a technique based on the vibrations of the atoms of a molecule. An infrared spectrum is commonly obtained by passing infrared radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a vibration of a part of a sample molecule (131).

2.4 Methods

2.4.1 Analysis by Inductively coupled plasma optical emission spectroscopy (ICP-OES)

Stones were collected from 20 Sudanese patients , The stones were collected , washed , dried and powdered to homogenous mixture and transferred into plastic containers. (0.5 g) was placed into digestive vial and 9ml of aquaregia (6ml HCl conc. and 2 mL HNO3 conc.) was added. the volume was then adjusted to 25 ml by adding water and the mixture was shaken and introduced the sample into the ICP-MS instrument and results.

2.4.2 Analysis by ultra violets spectrometer (UV)

The sample (0.5g) was placed into aquaregia (6 mL conc. HCl and 2 mL conc. HNO3) .were added. Water was added to a total volume of 25 ml. (1ml) of this

solution was taken and more deionizer water was added to total volume of 100ml.the spectra was then recorded.

2.4.3 Analysis by Fourier transforms infrared spectrometer (IR)

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

3-Results and Discussion

Elemental analysis by Inductively Coupled Plasma mass spectrometry (ICP)

Elemental analysis was conducted by inductively coupled plasma mass spectrometry (ICP) .The concentrations of 24 metals (part per million –ppm) in kidney stones and gall stones. According to our experimental results kidney stones (1-10) and gall stones (11-20) were distinguished they were sharply differing in the composition from the average element. Concentration in the samples collected from Sudanese patients determined by inductively coupled plasma mass spectrometry (ICP).were shown in tables (3-1) and (3-2). The results of elements concentration of different kidney stones and gall stones samples gave indication to concentration of elements.

3.1.1 Kidney stone

Table 3: 1 content of elements (ppm) in kidney stone sample

Elements	1	2	3	4	5	6	7	8	9	10
Ca	2.648	154.800	45.370	85.370	156.500	197.400	40.040	1.240	19.480	201.000
Ag	<0.0035	<0.0035	<0.0035	<0.0035	<0.0035	<0.0035	<0.0035	<0.0035	<0.0035	<0.0035
Al	75.6	41.79	77.70	19.71	15.36	<0.0055	<0.0055	508.6	<0.0055	153.3
As	<0.0281	<0.0281	<0.0281	<0.0281	<0.0281	<0.0281	<0.0281	<0.0281	<0.0281	<0.0281
Ba	0.1482	12.93	4.584	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Cd	<0.0009	0.33446	<0.0009	<0.0009	<0.0009	<0.0009	<0.0009	<0.0009	<0.0009	0.36302
Co	<0.0018	0.53734	0.47542	<0.0018	0.80848	<0.0018	0.4143	0.1552	<0.0018	<0.0018
Cr	0.4294	0.2185	0.3757	0.0811	0.3254	<0.0013	<0.0013	2.865	<0.0013	0.24882
Cu	7.044	<0.0013	4.013	3.251	3.285	22.52	10.72	2.369	6.990	<0.0013
Fe	23.65	36.90	139.9	467.4	30.26	26.94	15.79	381.5	16.75	48.07
K	424.2	1559	784.1	200.1	254.1	470.6	321.5	243.7	362.6	175.7
Mg	104.2	363.83	146.9	155.5	404.0	220.9	34.34	<0.0164	7.819	258.3
Mn	0.5498	0.5483	1.067	0.6850	0.1200	<0.0034	<0.0034	<0.0034	<0.0034	<0.0034
Mo	<0.0051	<0.0051	<0.0051	<0.0051	<0.0051	<0.0051	<0.0051	<0.0051	<0.0051	<0.0051
Na	11.08	300.1	215.5	36.49	69.20	62.09	8.276	3.312	11.03	35.15
Ni	5.507	6.350	12.03	0.2940	0.0966	<0.0053	<0.0053	5.240	<0.0053	<0.0053
Pb	<0.0150	6.983	5.117	2.354	2.890	<0.0150	<0.0150	<0.0150	6.772	6.635
Sb	6.081	10.0006	<0.0149	<0.0149	<0.0149	<0.0149	<0.0149	<0.0149	<0.0149	7.484
Se	29.42	<0.0266	<0.0266	86.39	<0.0266	<0.0266	<0.0266	<0.0266	<0.0266	22.12
Si	8.806	94.18	183.5	165.1	658.9	8.622	12.49	2.606	<0.0086	<0.0086
Sr	0.8206	216.3	30.38	22.04	85.34	<0.0005	<0.0005	13.09	<0.0005	109.6
Ti	0.3788	<0.0006	16.28	14.33	0.9472	<0.0006	<0.0006	<0.0006	<0.0006	<0.0006
V	0.4434	7.588	2.037	4.679	8.348	10.94	2.188	0.4961	0.803	10.86
Zn	19.27	401.1	41.69	26.26	35.13	18.32	<0.0022	<0.0022	<0.0022	105.4

3.1.2 Gall stones

Table 3: 2content of elements (ppm) in gall stone samples

4	11	12	13	14	15	16	17	18	19	20
Ca	52.660	29.310	1.371	732	68.620	15.990	88.980	201.000	17.710	141.300
Ag	035<0.0	035<0.0	035<0.0	035<0.0	035<0.0	035<0.0	035<0.0	035<0.0	035<0.0	035<0.0
Al	17.89	4.834	53.94	12.82	055<0.0	33.73	49.16	262.3	055<0.0	56.45
As	281<0.0	281<0.0	281<0.0	281<0.0	281<0.0	281<0.0	281<0.0	281<0.0	281<0.0	281<0.0
Ba	4.354	005<0.0	1.569	1.458	2.881	005<0.0	005<0.0	005<0.0	005<0.0	005<0.0
Cd	009<0.0	009<0.0	009<0.0	009<0.0	009<0.0	009<0.0	009<0.0	009<0.0	009<0.0	009<0.0
Co	0018<0.	018<0.0	018<0.0	018<0.0	018<0.0	018<0.0	018<0.0	018<0.0	018<0.0	018<0.0
Cr	0.2920	0.1937	4.638	013<0.0	013<0.0	0.1680	5.298	3.316	3.693	1.278
Cu	35.69	545.6	8.776	2.893	4172	723.0	464.3	151.9	483.8	1438
Fe	20.93	54.61	96.75	37.14	1418	189.2	280.5	145.7	175.8	325.1
K	318<0.0	318<0.0	318<0.0	163.8	399	458.0	47.64	82.38	155.8	386.3
Mg	354.0	247.7	45.37	35.11	1021	631.5	927.7	837.4	198.7	2337
Mn	143.7	149.6	4.117	0.3205	424.3	139.5	1131	121.1	57.4	3025
Mo	051<0.0	051<0.0	051<0.0	051<0.0	051<0.0	051<0.0	051<0.0	051<0.0	051<0.0	051<0.0
Na	151.2	254.3	19.57	48.99	277.6	269.0	234.3	172.1	355.6	449.1
Ni	053<0.0	1.113	4.259	6.511	053<0.0	1.474	1.154	1.364	053<0.0	053<0.0
Pb	150<0.0	150<0.0	1.424	5.733	38.204	150<0.0	9.068	150<0.0	12.67	13.76
Sb	149<0.0	149<0.0	149<0.0	149<0.0	149<0.0	149<0.0	149<0.0	149<0.0	17.64	4.032
Se	266<0.0	266<0.0	266<0.0	266<0.0	266<0.0	266<0.0	266<0.0	266<0.0	266<0.0	119.1806
Si	086<0.0	086<0.0	086<0.0	086<0.0	086<0.0	086<0.0	086<0.0	086<0.0	086<0.0	086<0.0
Sr	12.51	380.43	3.052	005<0.0	005<0.0	9.255	103.6	399.3	10.41	119.5
Ti	006<0.0	006<0.0	6.375	10.968	006<0.0	36.49	683.4	006<0.0	006<0.0	006<0.0
V	2.764	1.457	0.2540	0.3797	3.217	0.7364	5.924	11.45	0.8037	7.398
Zn	10.98	36.18	27.22	4.137	214.5	91.23	59.72	37.58	147.9	187.1

Content of barium was higher in some samples (2, 3, 11 and 15). As we can see from figure 3.1

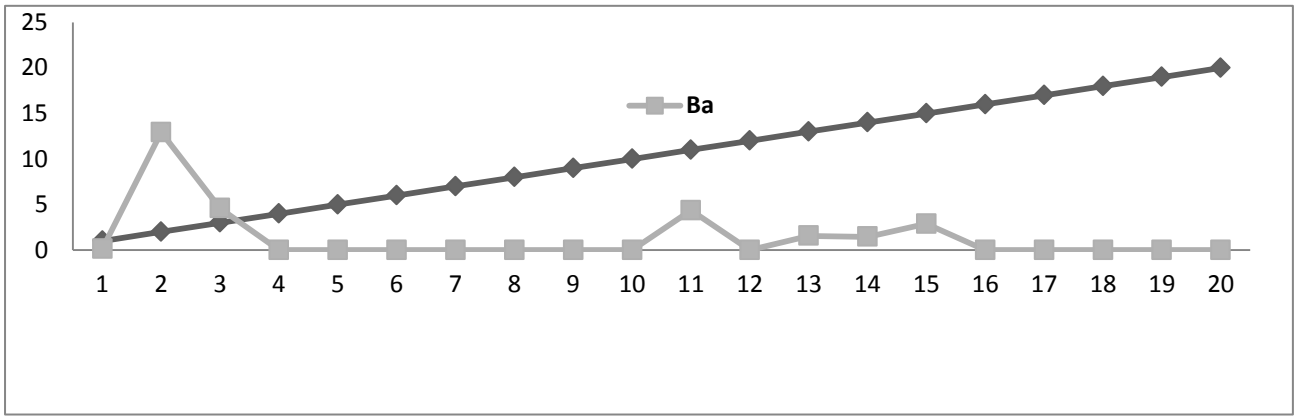


Figure 3.1: Ba^{2+} content (ppm) by ICP in samples 1-20

It has been found that these stones contain mostly calcium and was considered as the main constituent of stones of all different types. It is obvious that Ca content is affected by the type of food and drinks patient take, including diary and milk products, eggs, tea, and hard water. Samples number (2, 5, 6, 10, 14, 18 and20) shows increase in Ca element. Such as figure3.2

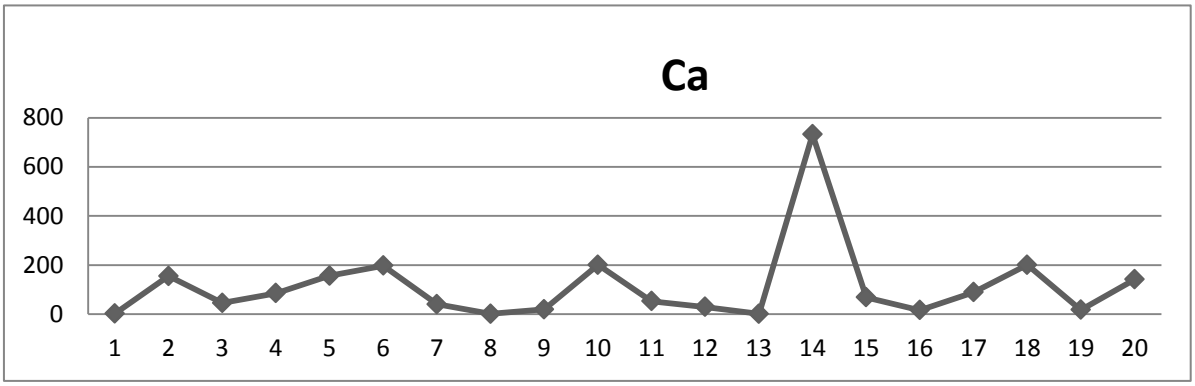


Figure 3.2: Ca^{2+} content (ppm) by ICP in samples 1-20

Samples number (8, 13, 17, 18, 19 and 20) shows increase in Cr. Although Cr in all sample low concentration. As figure 3.3

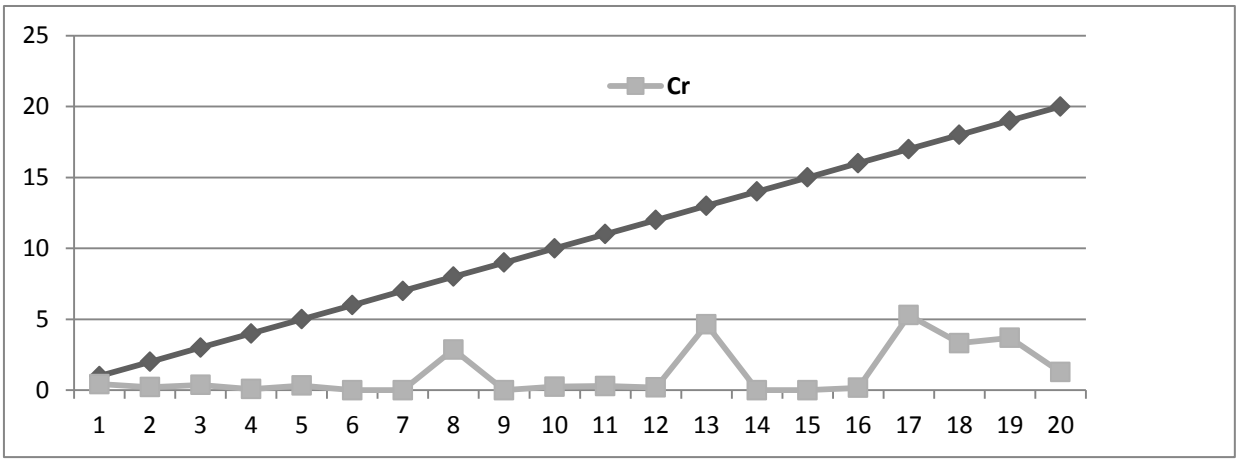


Figure 3.3: Cr^{+3}content (ppm) by ICP in samples 1-20

Concentration of transition metal ions such as Cu was high in gall stones samples (8, 13, 17, 18, 19and20). And Lower in kidney stones samples as figure 3.4. Green foods, flour, milk products and meats were mainly responsible for these high concentrations. As we can see in figure 3.4

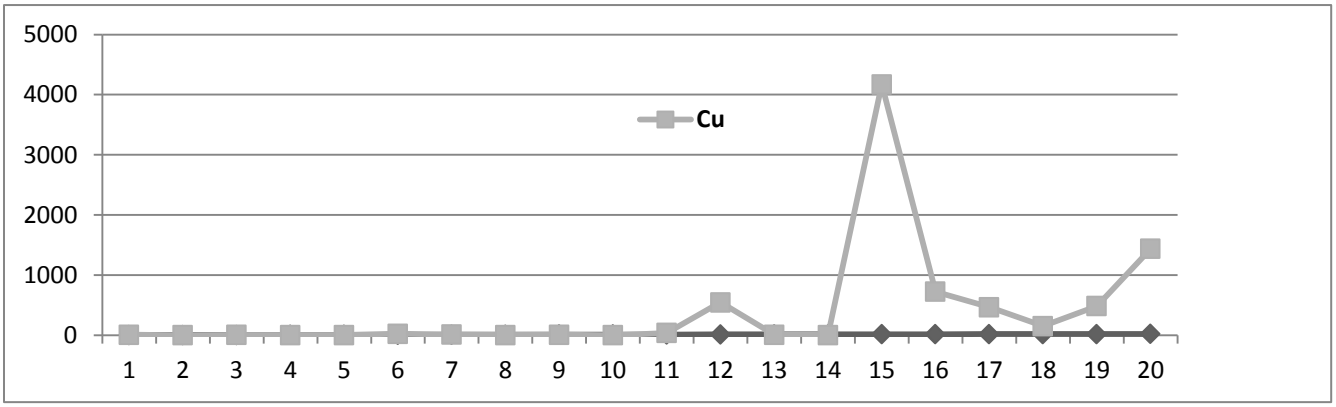


Figure 3.4: Cu^{+2} content (ppm) by ICP in samples 1-20

Concentration of Fe was low in all samples (kidney and gall stones). Except the highest concentrations were found in Samples number (3, 4,8,15, 17, 18, 19 and 20) .see figure 3.5

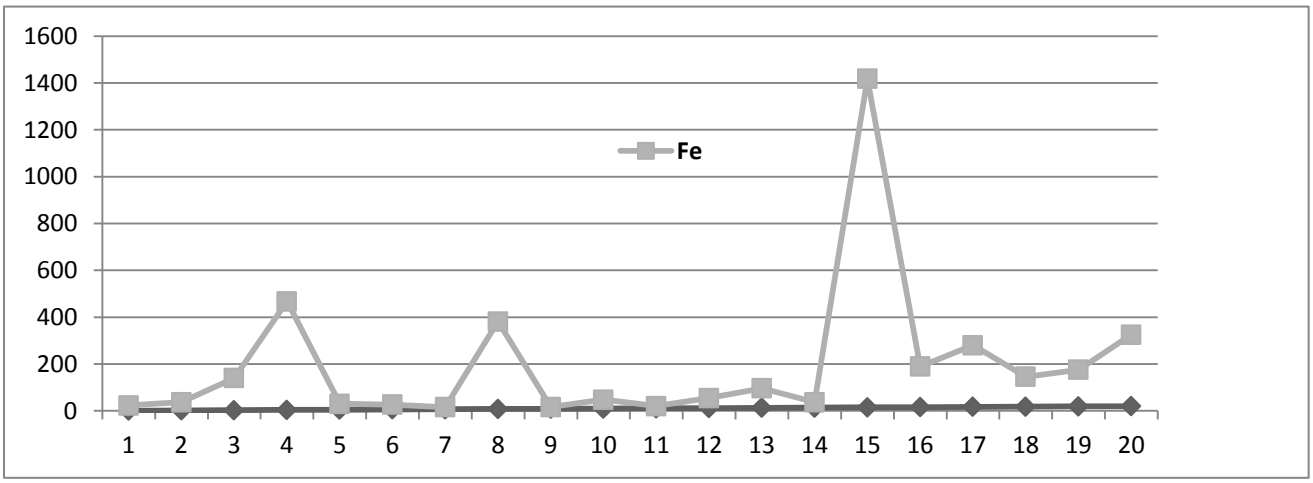


Figure 3.5: Fe^{2+}content (ppm) by ICP in samples 1-20

Potassium show increase in kidney stones samples (1-10) and present in low concentration in gall stones samples (11, 12, 13 and, 18) (as figure 3.6)

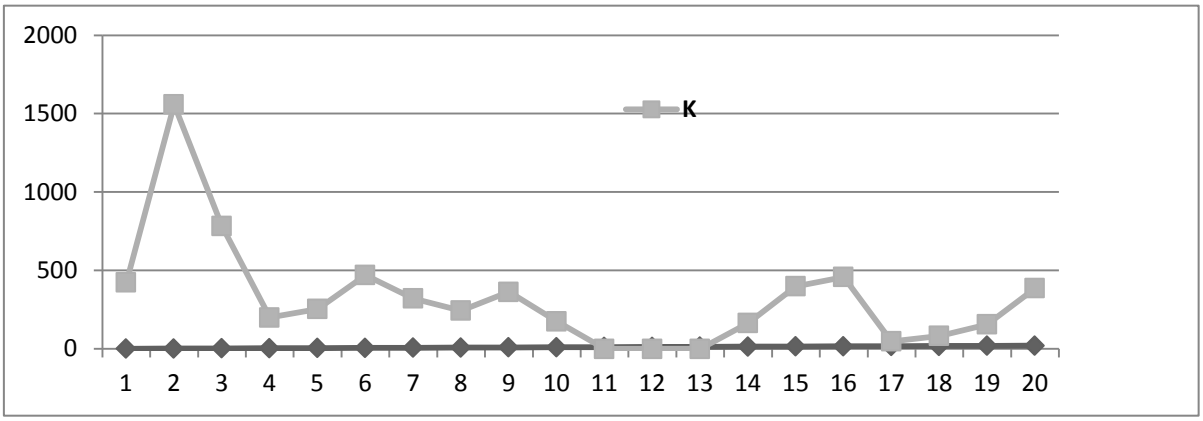


Figure 3.6: K^{1+}content (ppm) by ICP in samples 1-20

Samples number (2, 5,6,10, 11, 12, 15, 16, 17, 18 and20) shows increase in Mg element .see figure 3.7

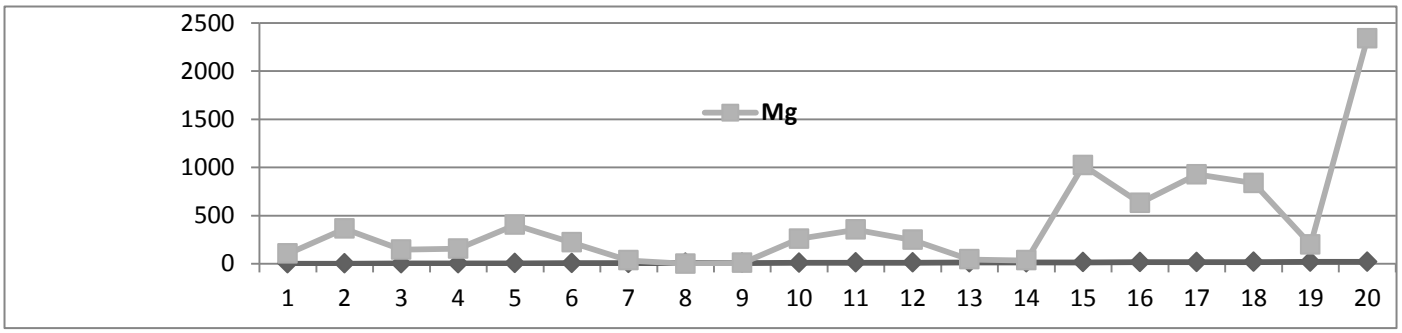


Figure 3.67mg content (ppm) by ICP in samples 1-20

Concentration of transition metal ions such as Mn was high in gall stones samples (15, 17and20) and Lower in kidney stones samples .Such as figure 3.8

Mn is present in pigment and mixed gallstones ranging between 16-1350 ppm. The main source of Mn is beans, tea, and green foods.

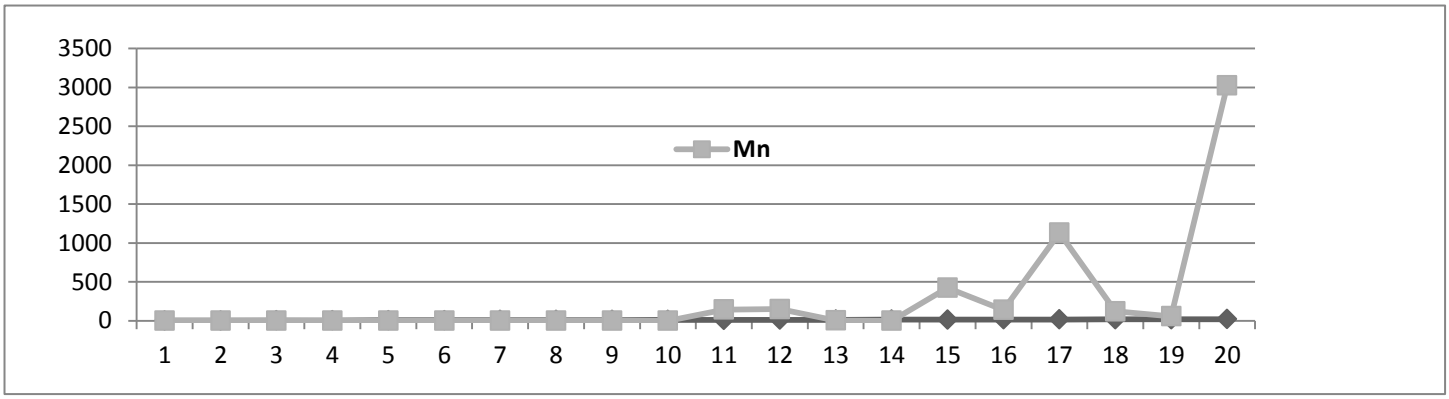


Figure 3.8: Mn^{2+}content (ppm) by ICP in samples 1-20

Na is high in gall than kidney. Samples number (2,3,5,6,11,12,15,16,17,18,19 and 20) shows increase in Na element. See Figure 3.9

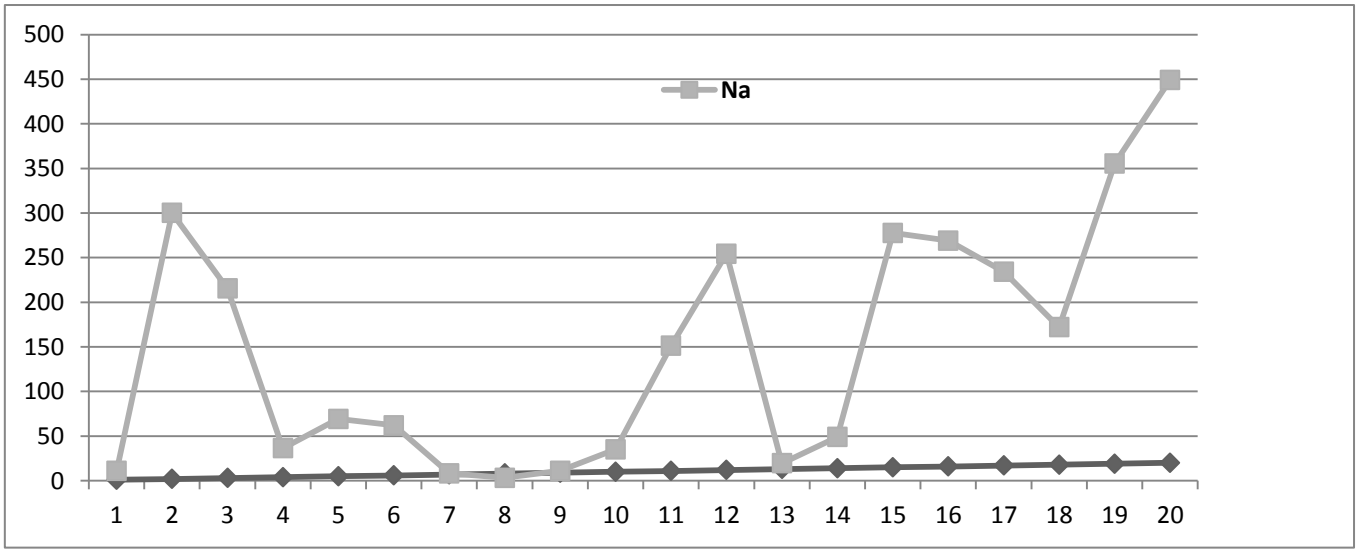


Figure 3.9:Na^{1+} content (ppm) by ICP in samples 1-20

Samples number (14, 16, 18and19) shows increase in Pb element (as figure 3.10

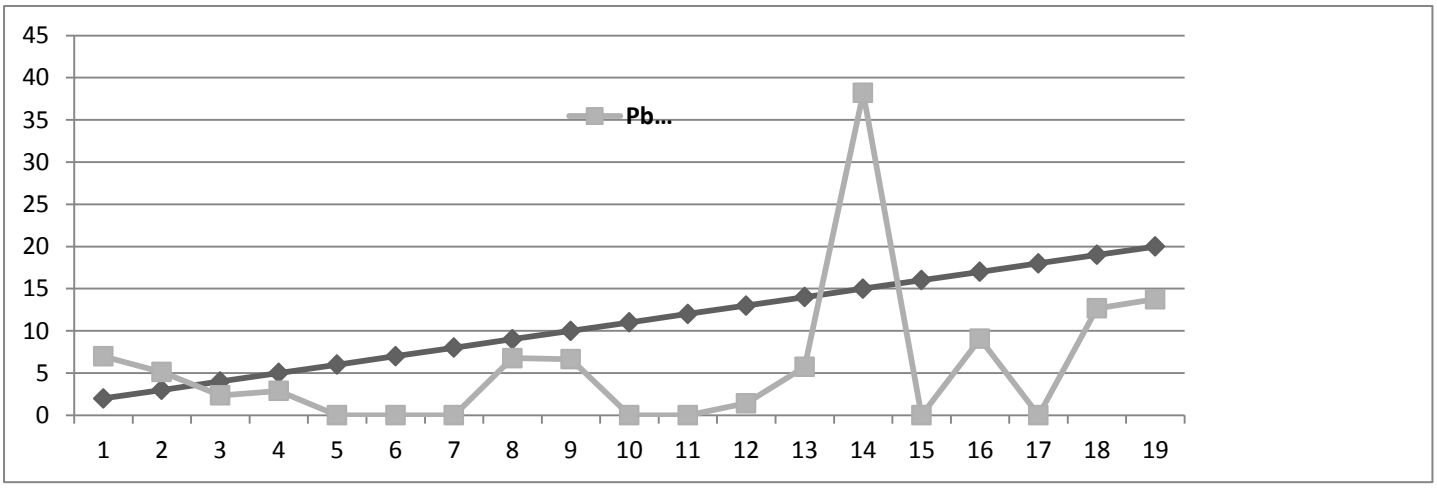


Figure 3.10: Pb^{+2}content (ppm) by ICP in samples 1-20

Samples number (1, 4, 10 and20) shows increase in Se element see figure 3.11

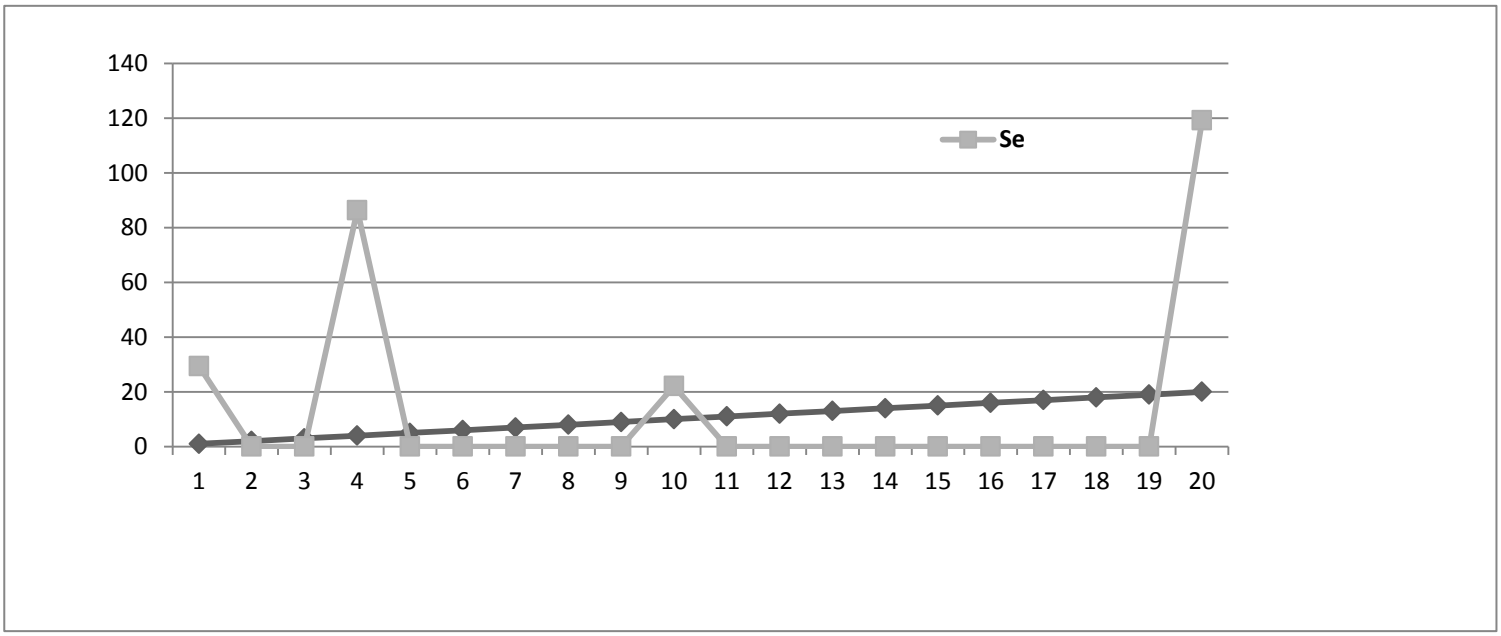


Figure 3.1 1: Se^{2+} content (ppm) by ICP in samples 1-20

Samples number (2, 3, 5, 10, 12, 17, 18 and 20) shows increase in Sr element. As show in figure 3.12

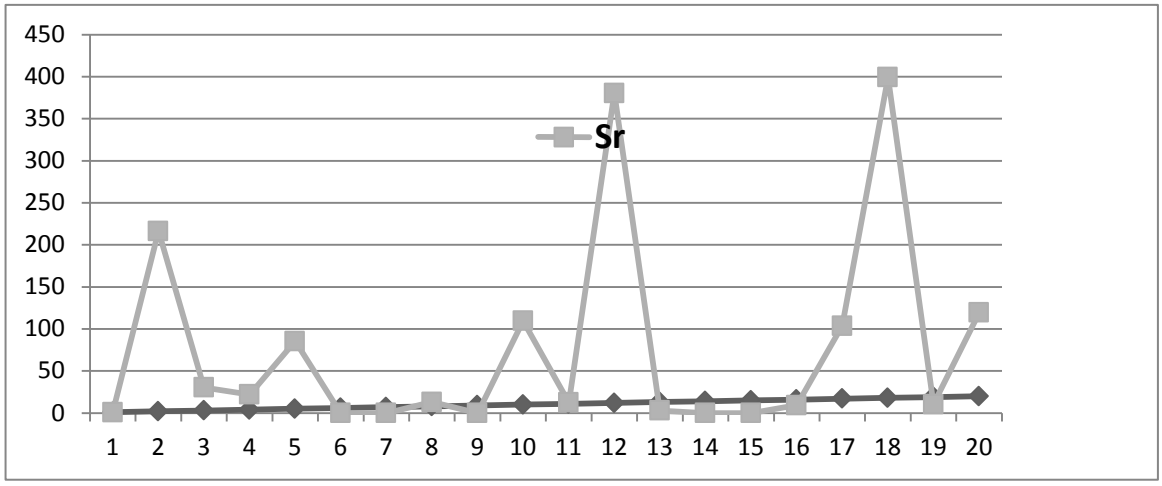


Figure 3.12: Sr^{+3}content (ppm) by ICP in samples 1-20

Samples number (2, 3, 5, 10, 15, 16, 17, 19 and20) shows increase in Zn element .see figure 3.13 high in kidney and gall stones.

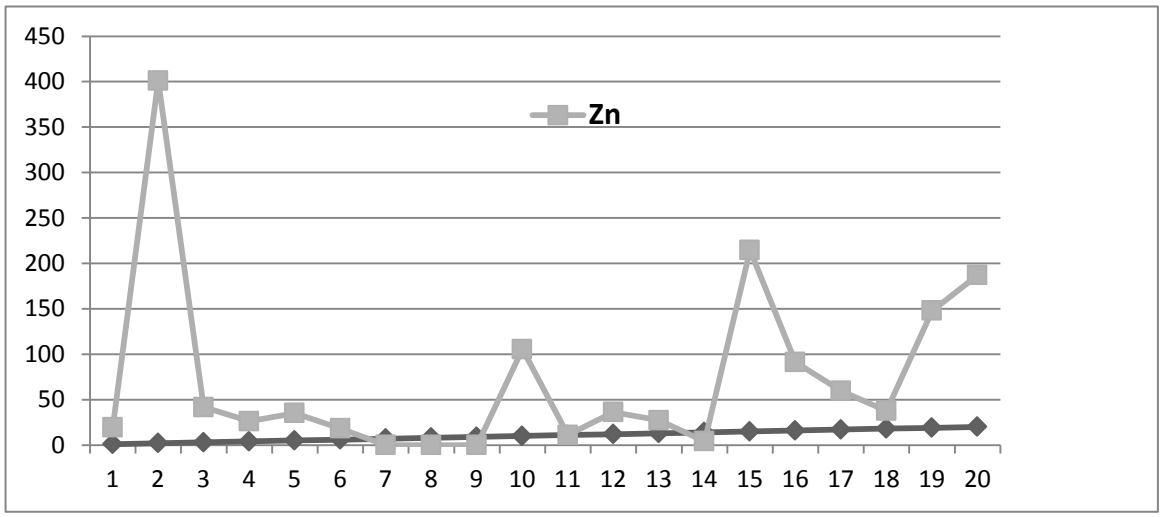


Figure 3.13: Zn^{+2} content (ppm) by ICP in samples 1-20

Samples number (1, 2, 3, 8, 10, 13, 18 and 20) shows increase in Al element (see figure 3.14

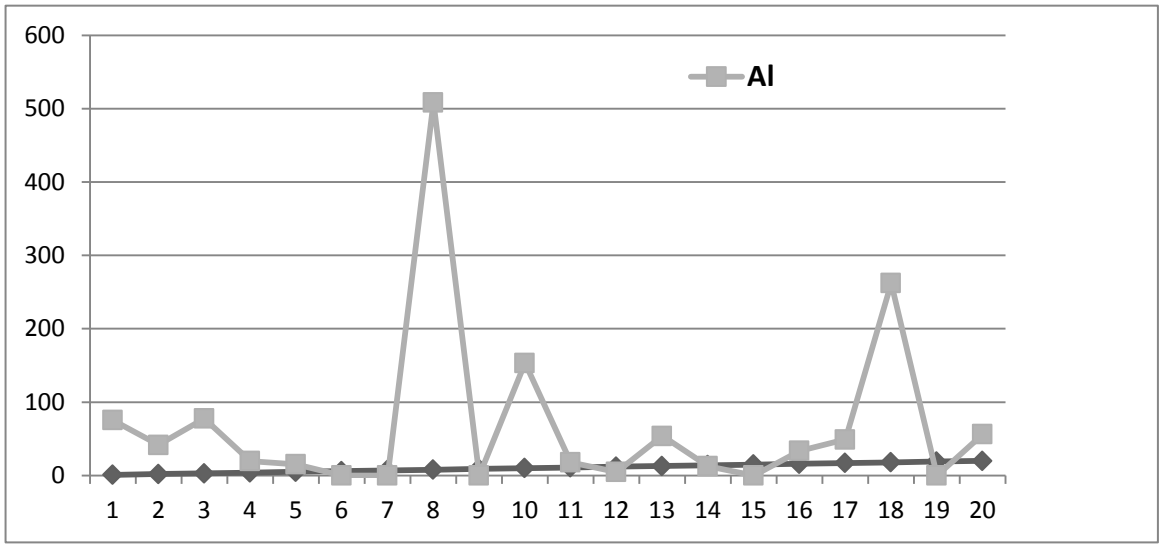


Figure 3.14: Al^{+3} content (ppm) by ICP in samples 1-20

The elements Cd, Co and Ti were contain low concentration in all samples. (See in figure 3.15, figure 3.16 and figure3.17)

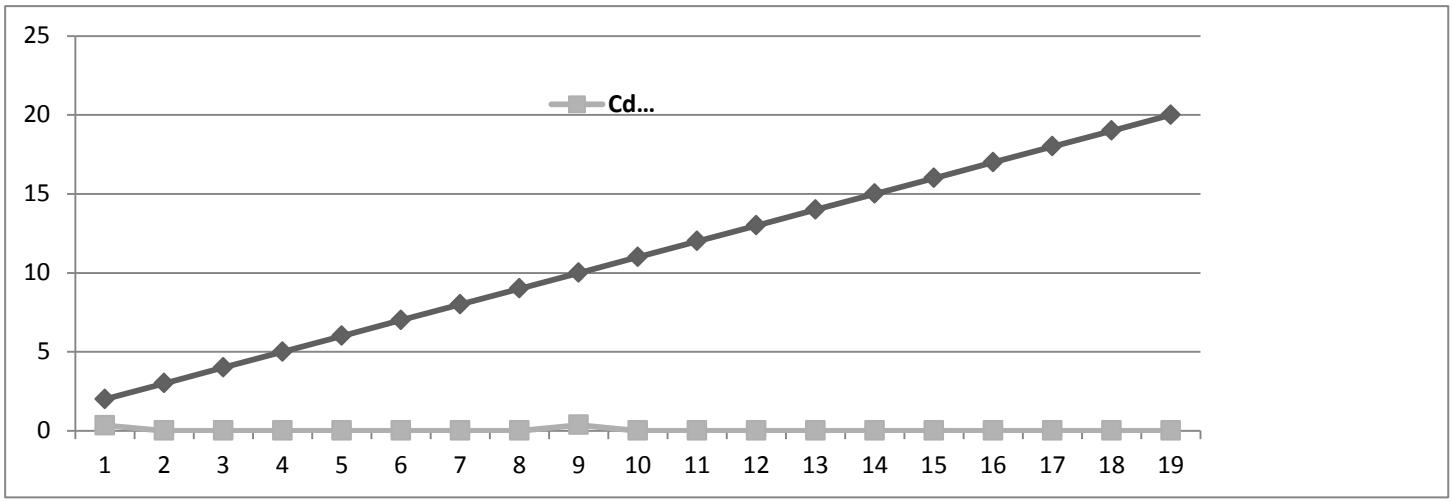


Figure 3.15 Cd^{+2} content (ppm) by ICP in samples 1-20

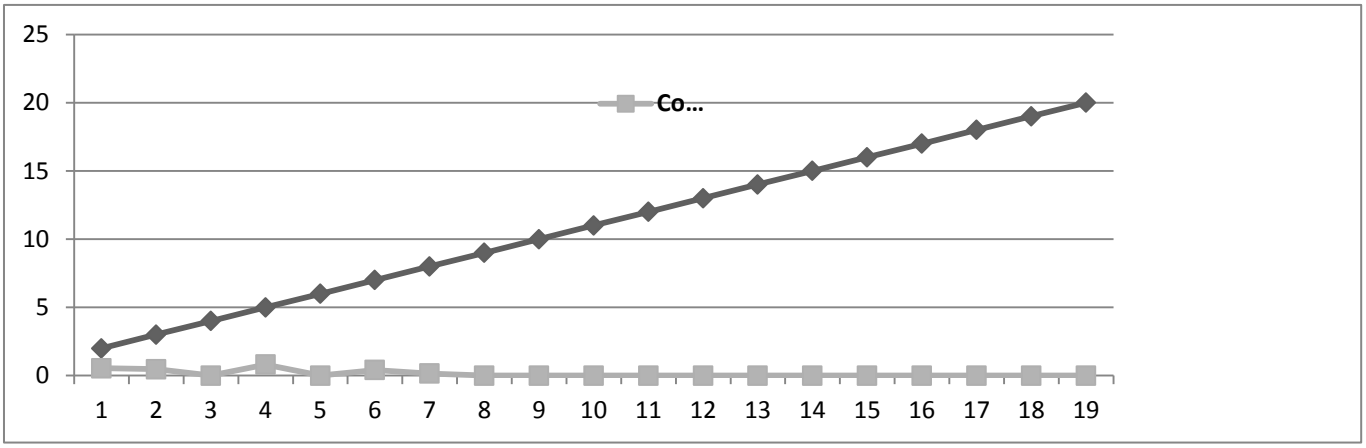


Figure 3.16: Co^{+2} content (ppm) by ICP in samples 1-20

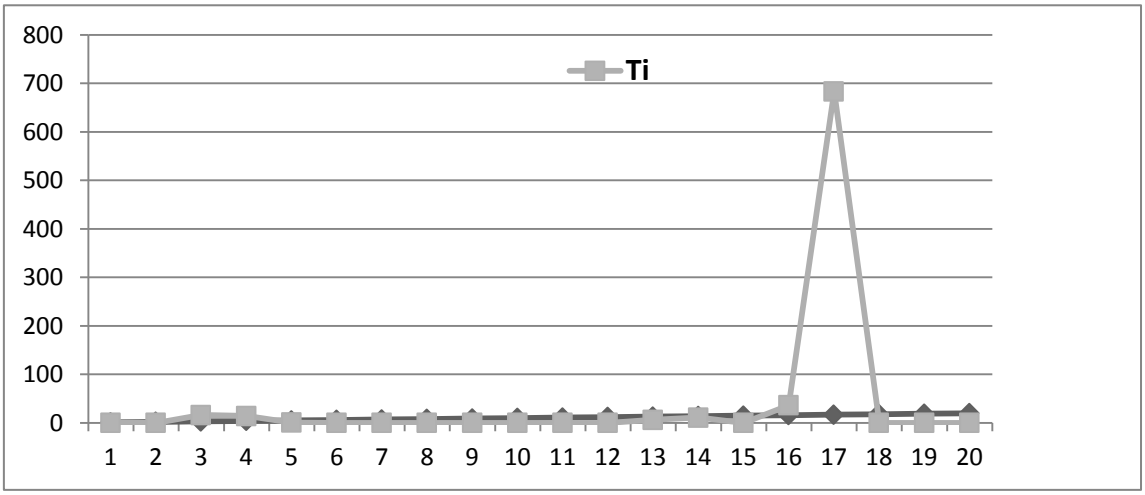


Figure 3.17: Ti^{+3} content (ppm) by ICP in samples 1-20

Samples number (1, 2, 3, 8,13and14) shows increase in Ni element seesfigu3.17

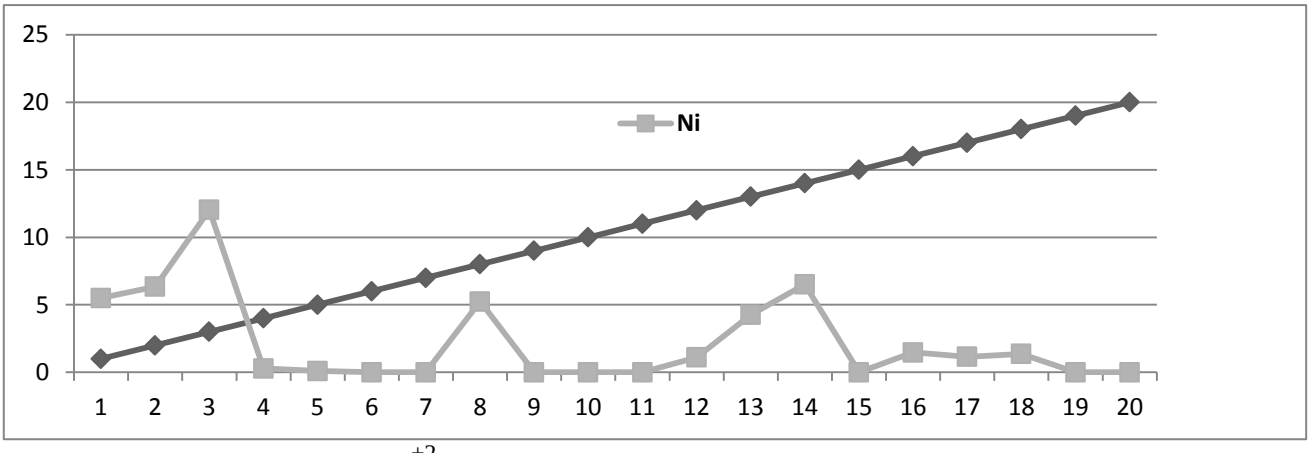


Figure 3.18: Ni $^{+2}$ content (ppm) by ICP in samples 1-20

Samples number (2, 4, 5, 6, 7, 10, 15, 17 ,18 and20) shows increase in V element see figure 3.19

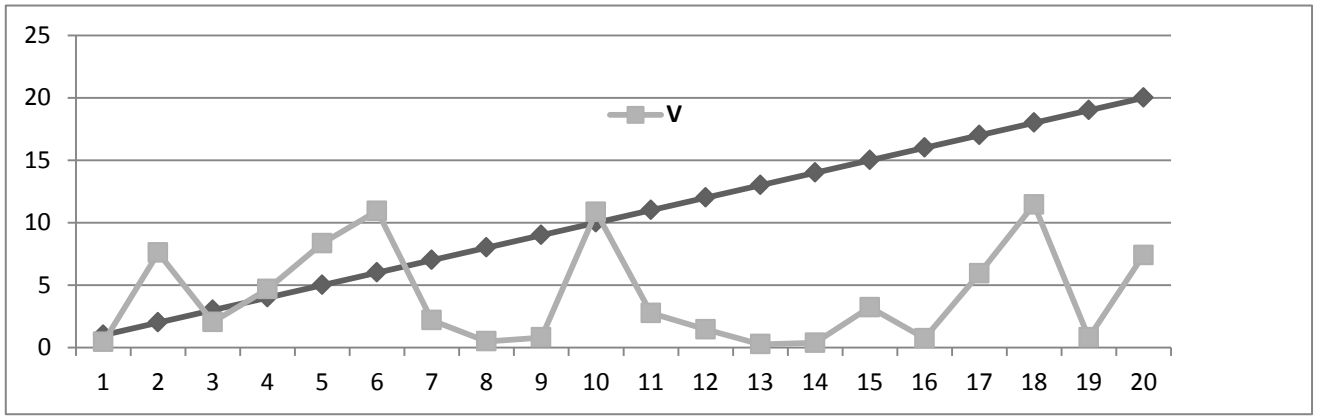


Figure 3.19: V content (ppm) by ICP in samples 1-20

Samples number (2, 3, 4 and 5) shows increase in Si element sees figure 3.20

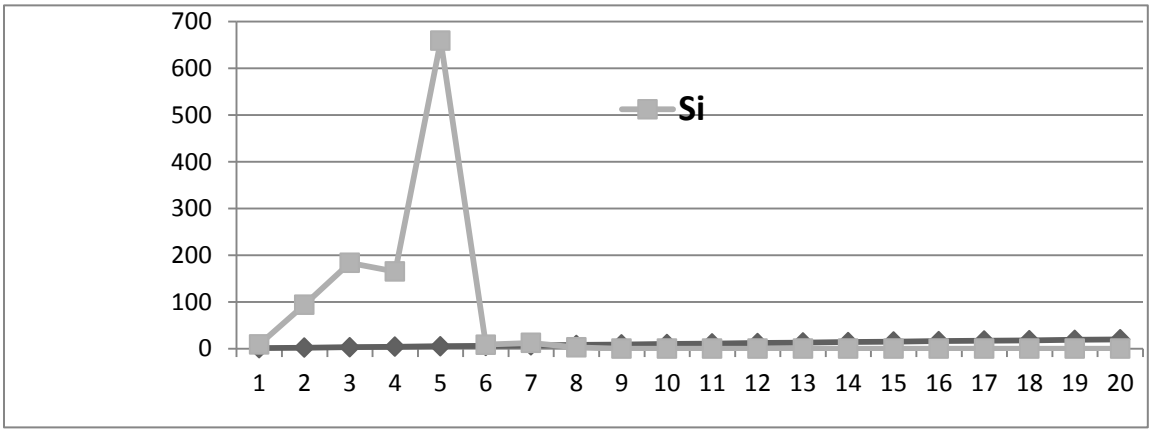


Figure 3.20: Si content (ppm) by ICP in samples 1-20

Samples number (1, 2, 10, 19and20) shows increase in Sb element (see figure 3.22)

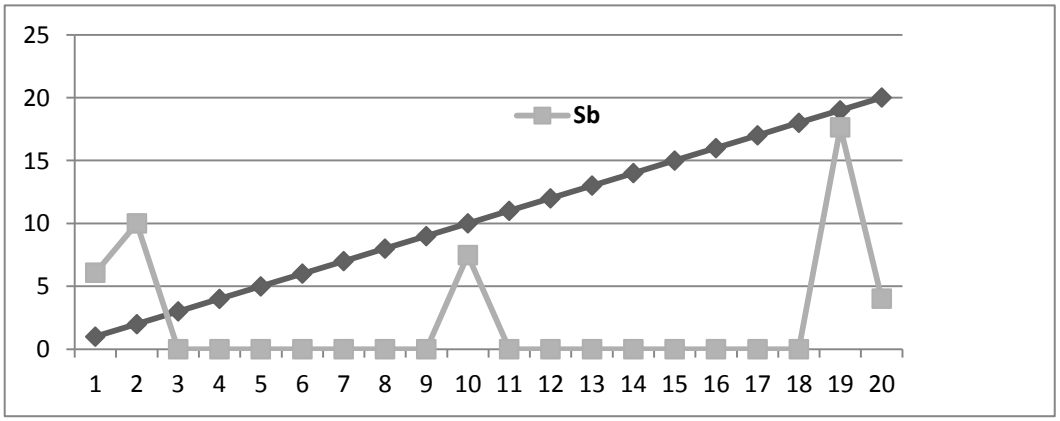


Figure 3.21 Sb^{+3}content (ppm) by ICP in samples 1-20

3.2 Analysis by Ultra violet spectrometer (1800-Shimadzu):

The wave length for all samples present in narrow range 230-205 nm and that attributed to presence of carbonyl group which absorb radiation at 220nm and have intensive absorbance ε=10,000 L cm^{-1} $mol^{-1}$and increase the value is attributed to rest of the molecule attached to carbonyl which differ from sample to another (135).

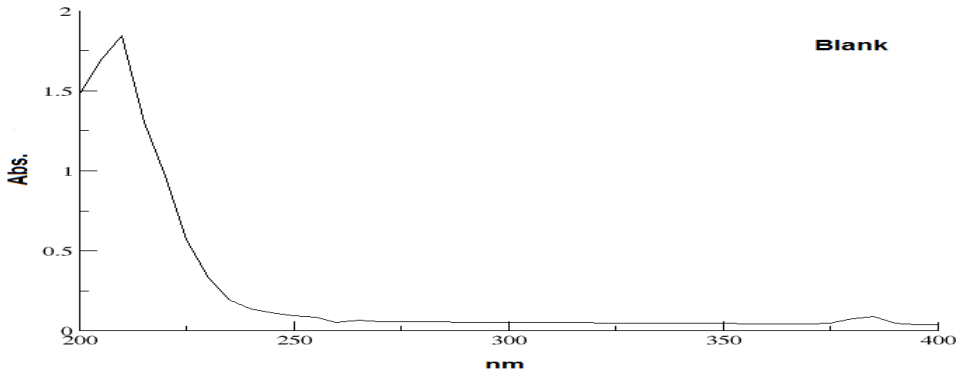


Figure 3.22 ultra violet spectrum of blank

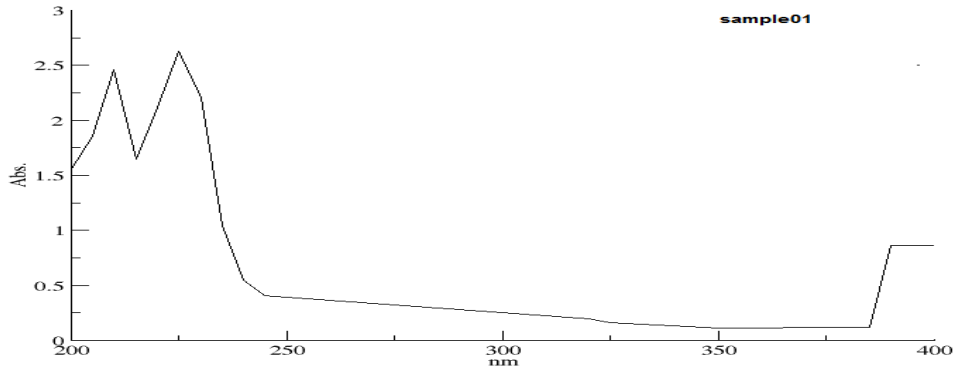


Figure 3.23 ultra violet spectrum of Sample (1)

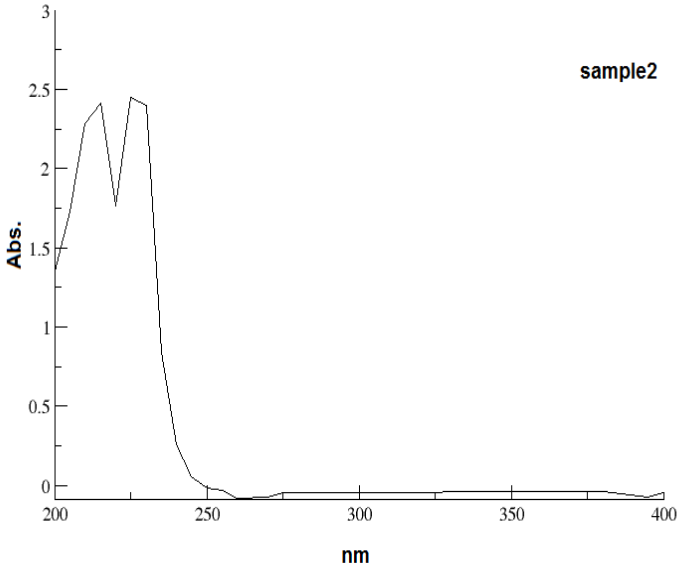


Figure 3.24 ultra violet spectrum of Sample (2)

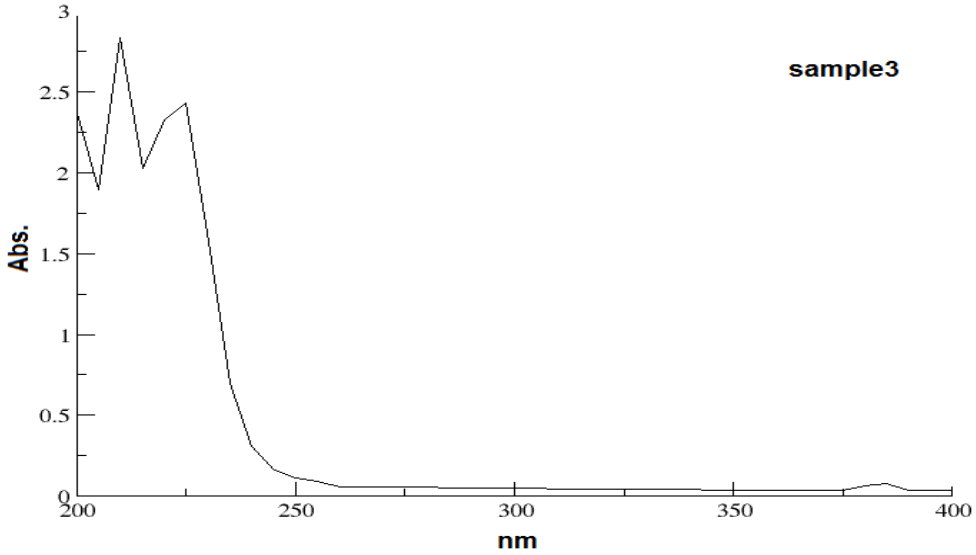


Figure 3.25 ultra violet spectrum of Sample (3)

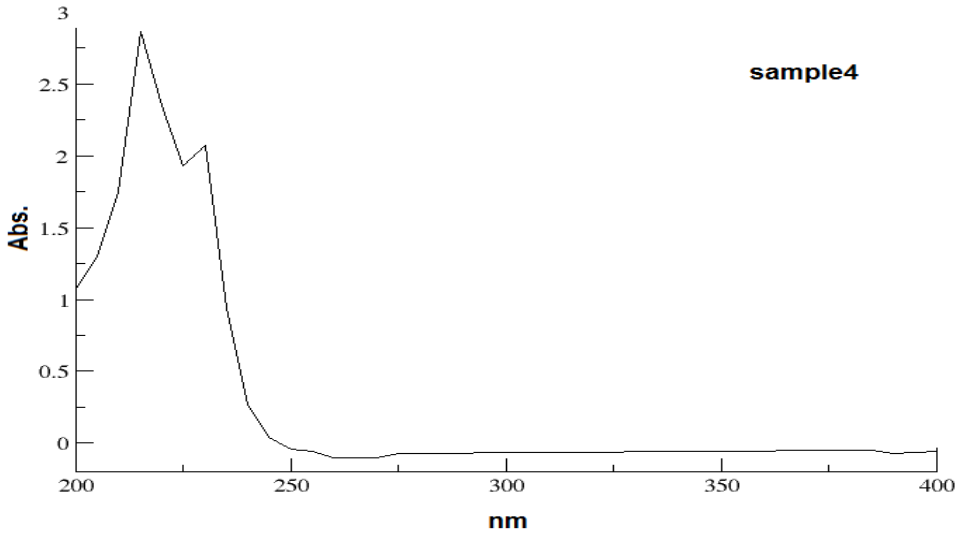


Figure 3.26 ultra violet spectrum of Sample (4)

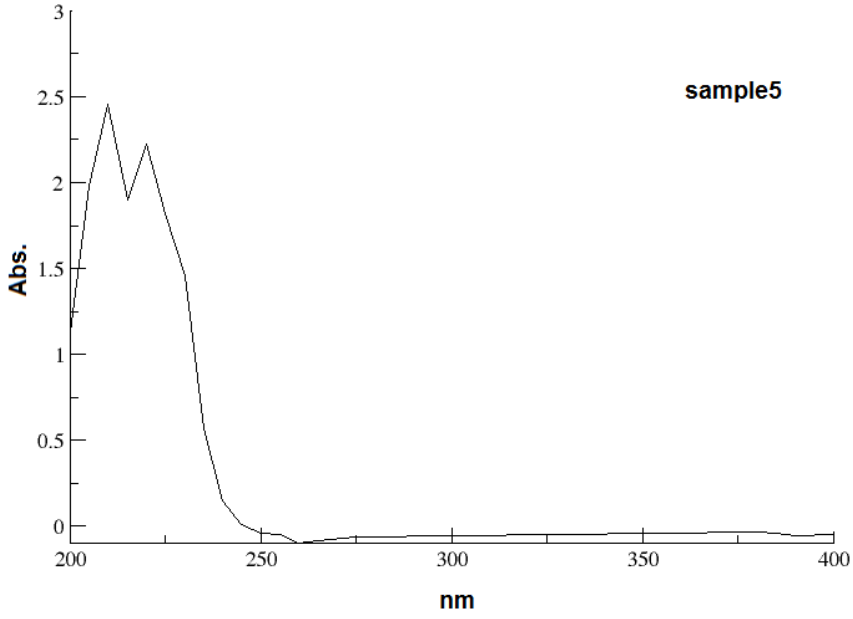


Figure 3.27 ultra violet spectrum of Sample (5)

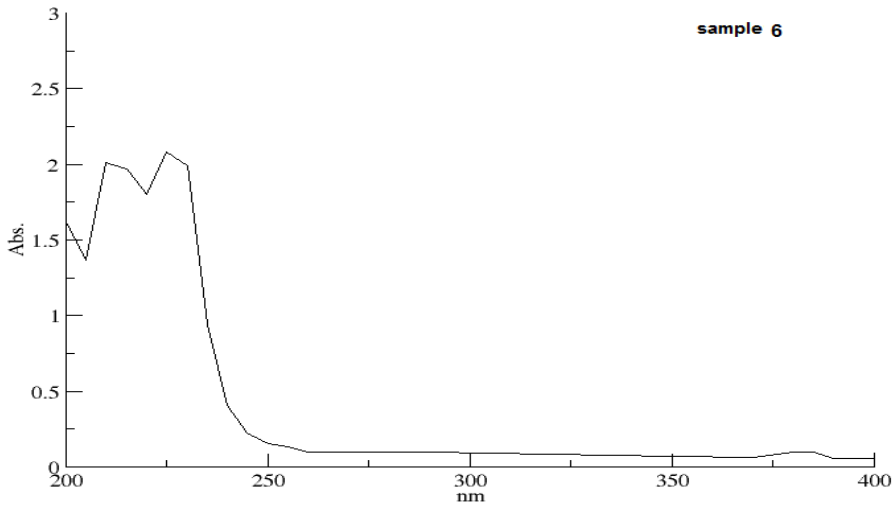


Figure 3.28 ultra violet spectrum of Sample (6)

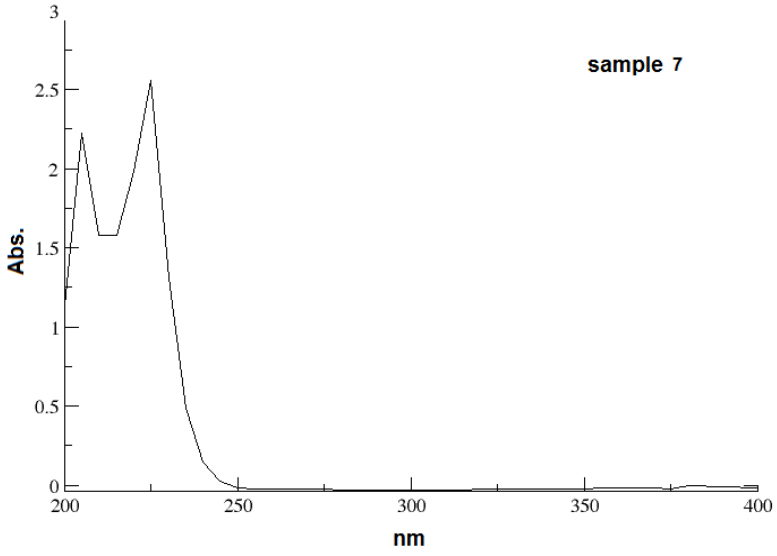


Figure 3.29 ultra violet spectrum of Sample (7)

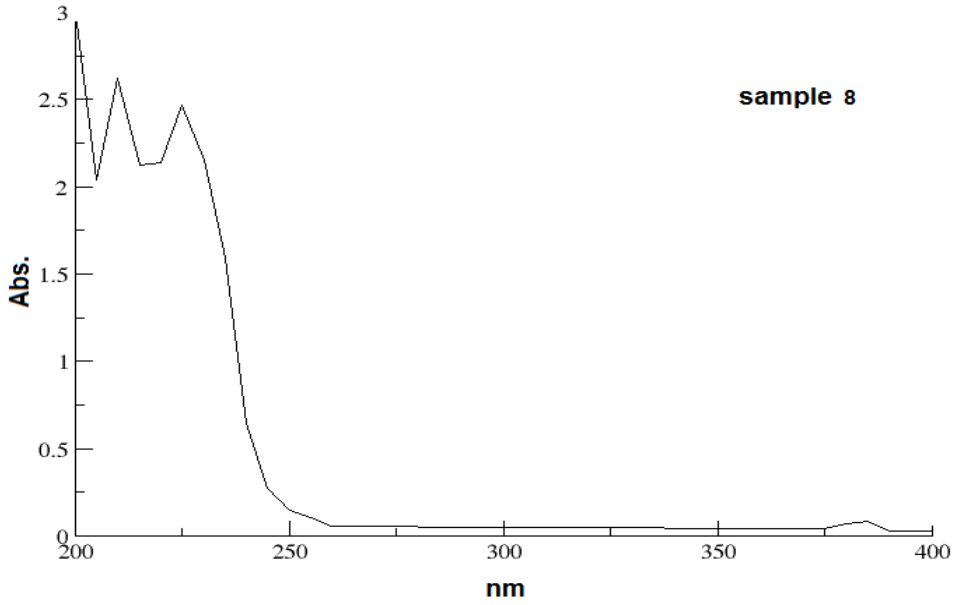


Figure 3.30 ultra violet spectrum of Sample (8)

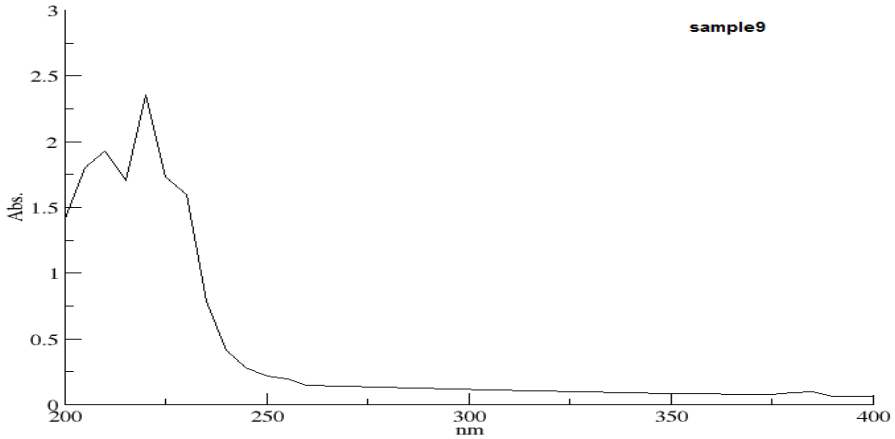


Figure 3.31 ultra violet spectrum of Sample (9)

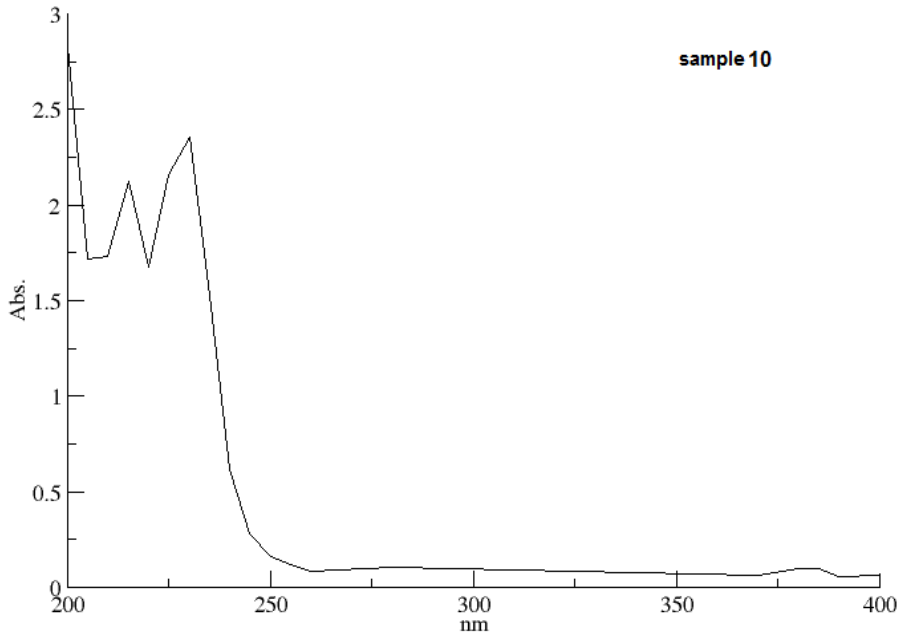


Figure 3.32 ultra violet spectrum of Sample (10)

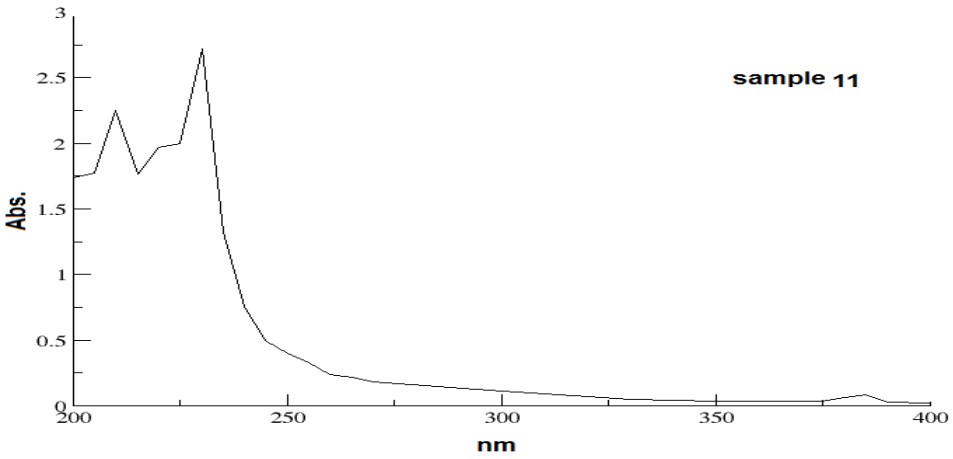


Figure 3:33 ultra violet spectrum of Sample (11)

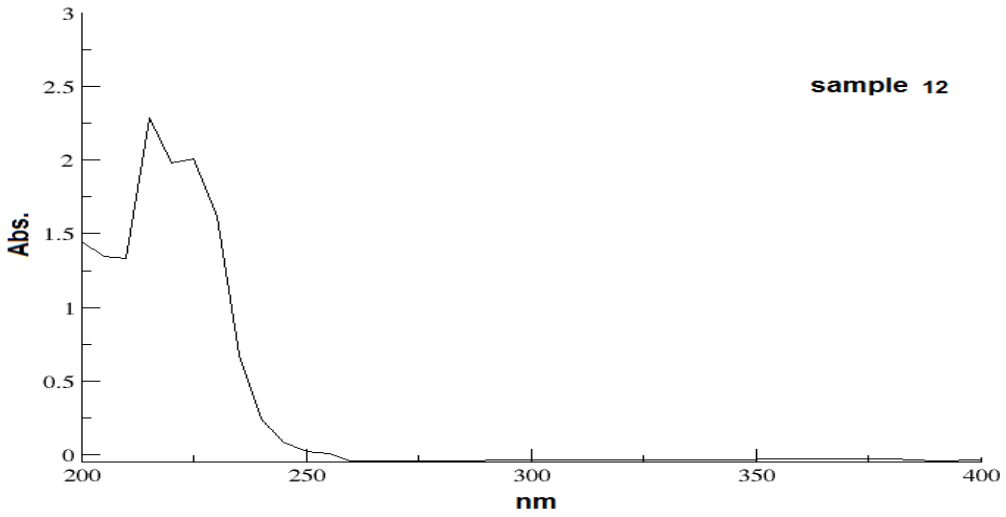


Figure 3.34 ultra violet spectrum of Sample (12)

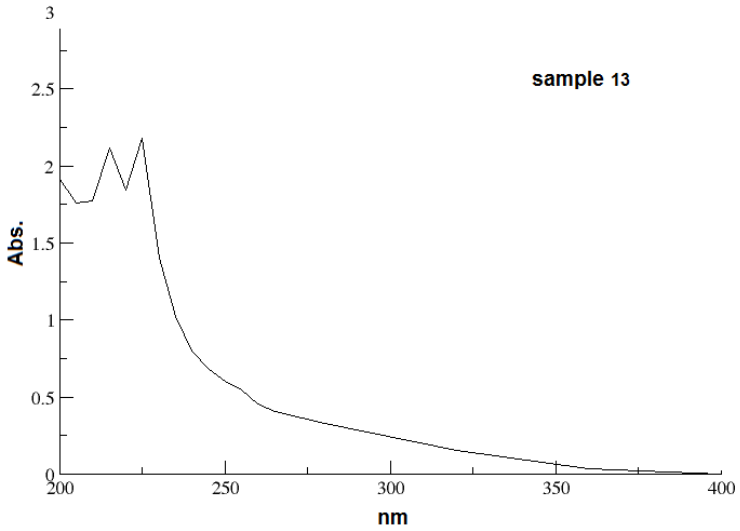


Figure 3.35 Ultra Violet Spectrum of Sample (13)

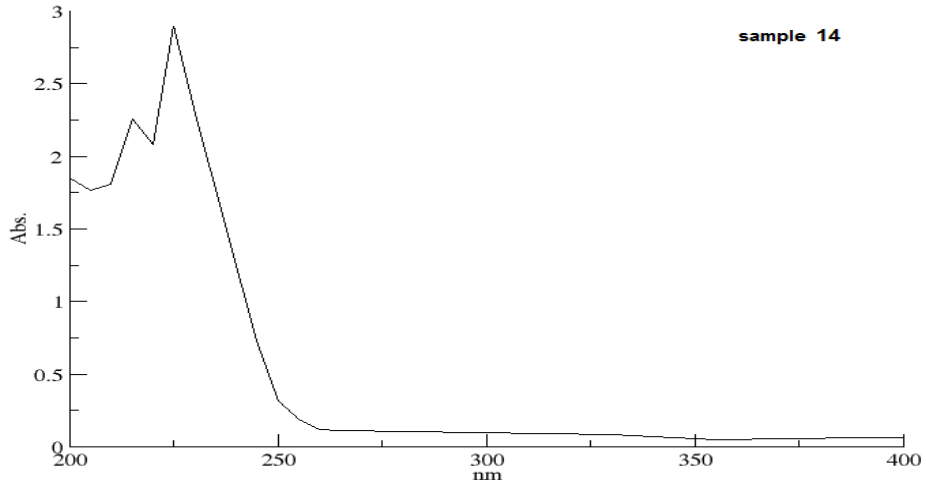


Figure 3.36 ultra violet spectrum of Sample (14)

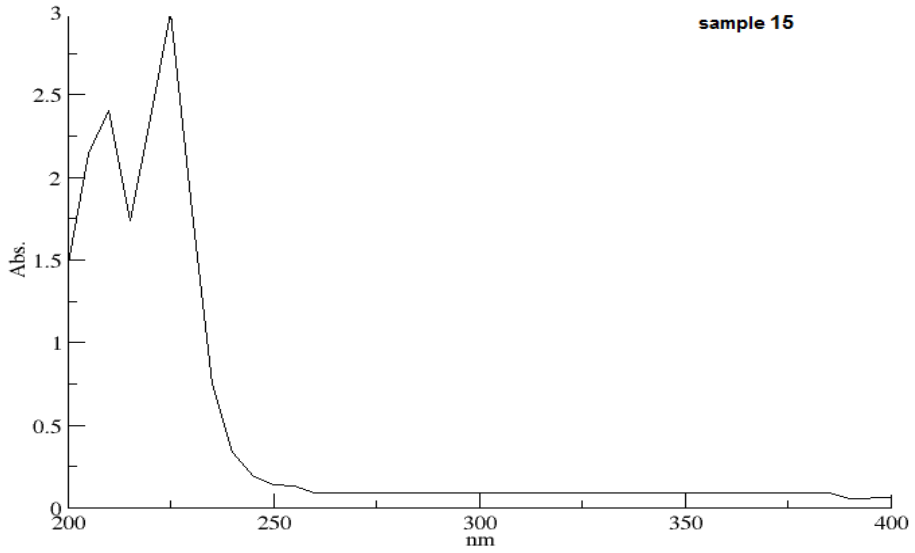


Figure 3.37 ultra violet spectrum of Sample (15)

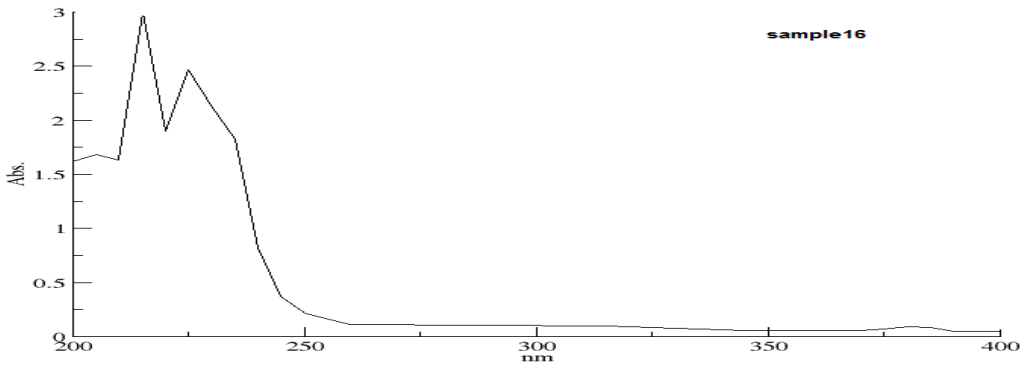


Figure 3.38 ultra violet spectrum of Sample (16)

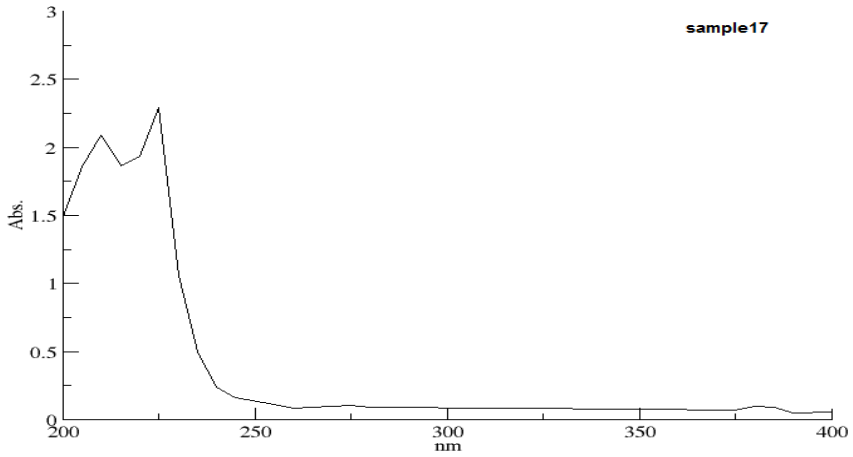


Figure 3.39 ultra violet spectrum of Sample (17)

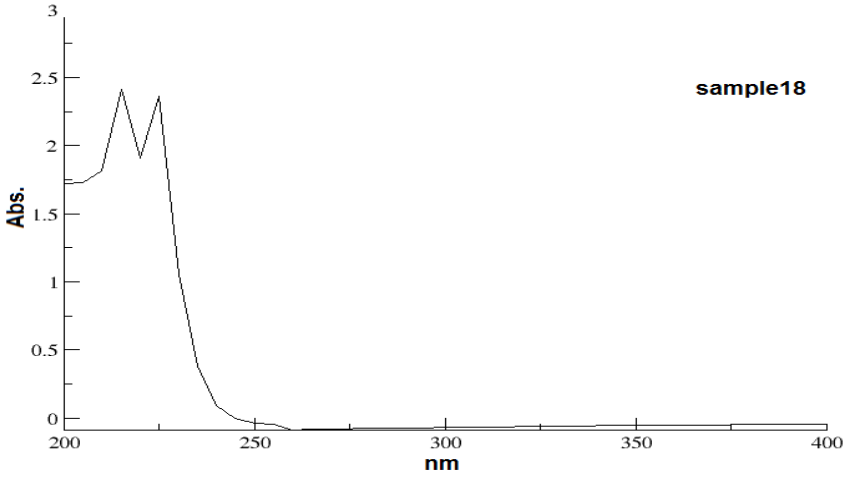


Figure 3.40 ultra violet spectrum of Sample (18)

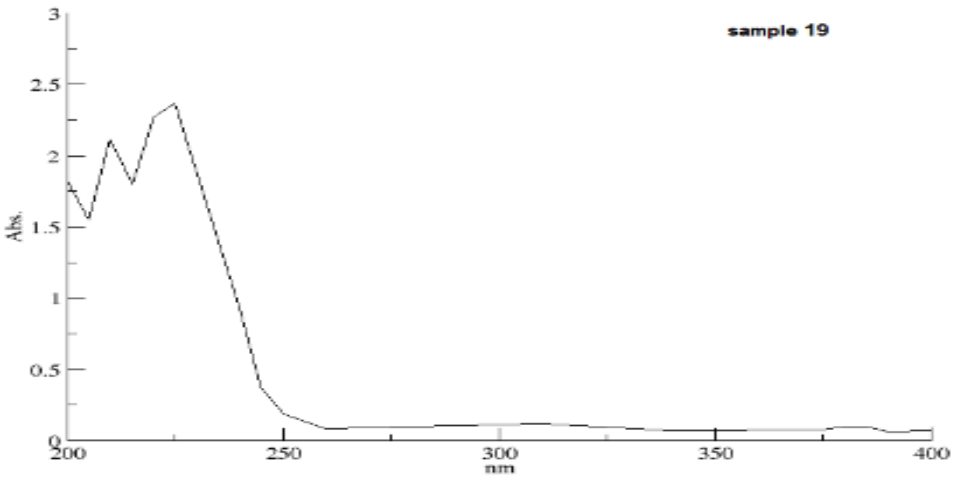


Figure 3.41 ultra violet spectrum of Sample (19)

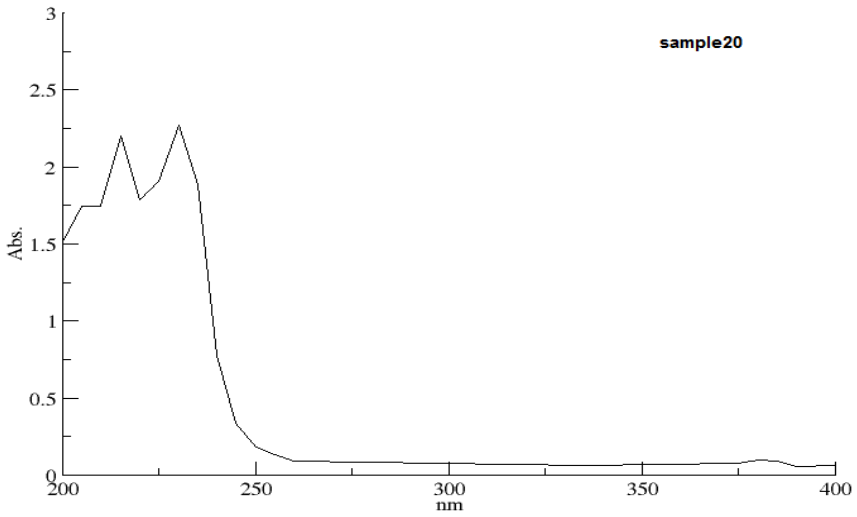


Figure 3.42 ultra violet spectrum of Sample (20)

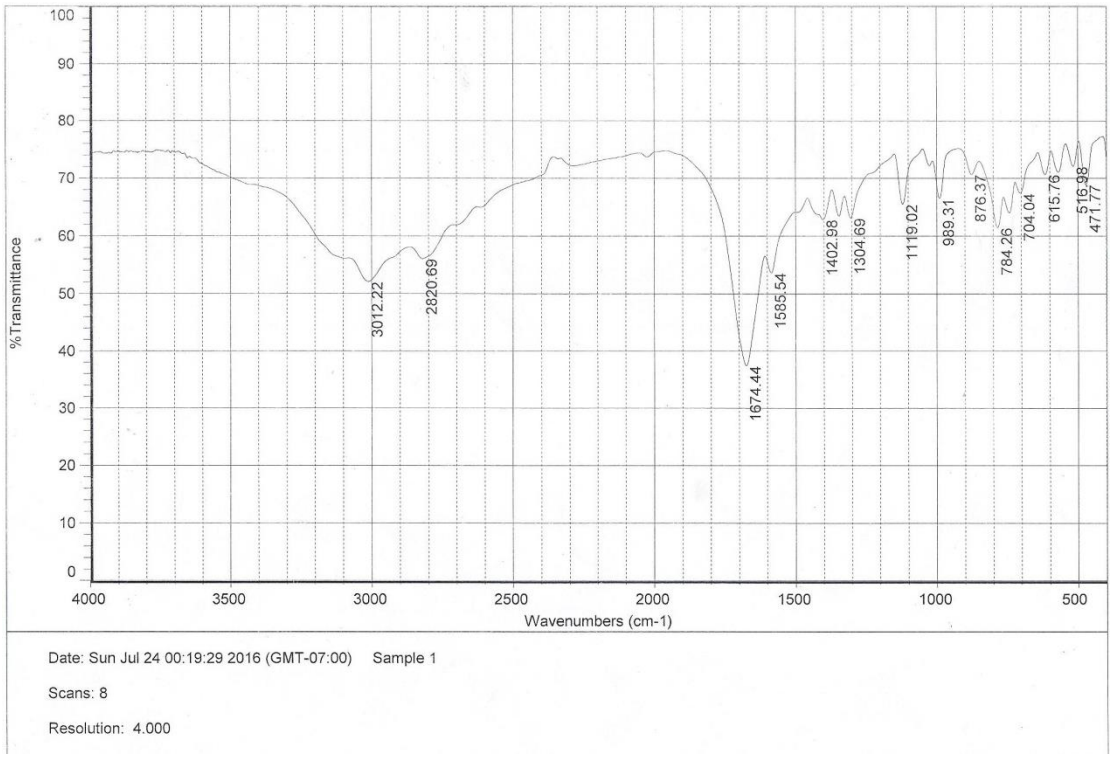


Figure 3.43 Infrared Spectrum of Sample (1)

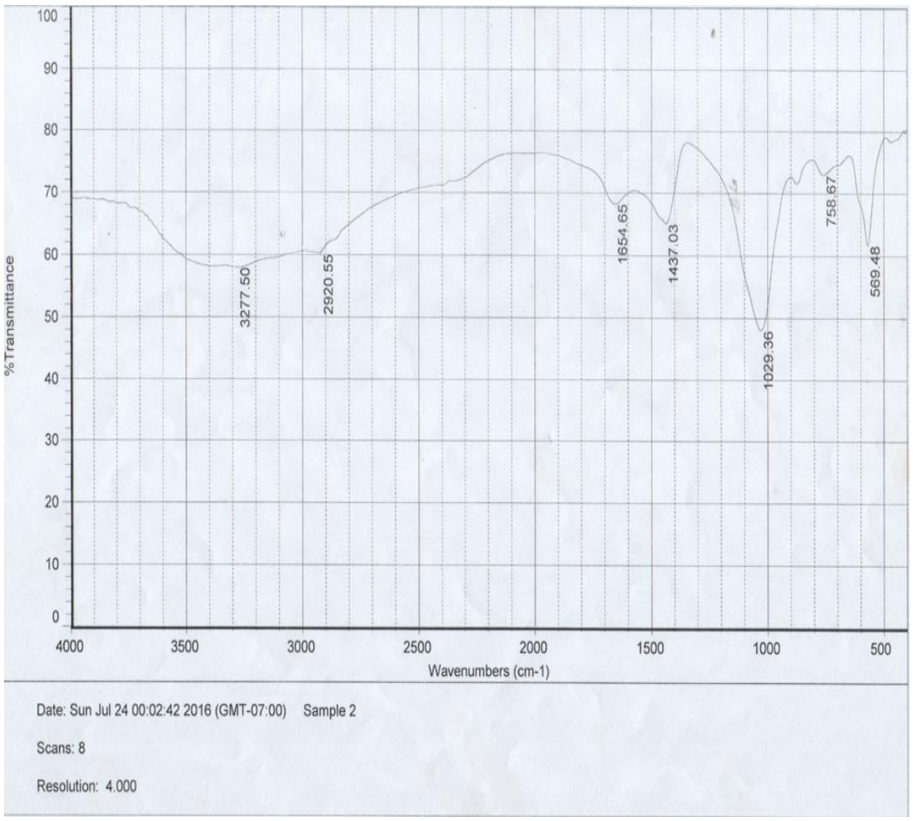


Figure 3.44 Infrared Spectrum of Sample (2)

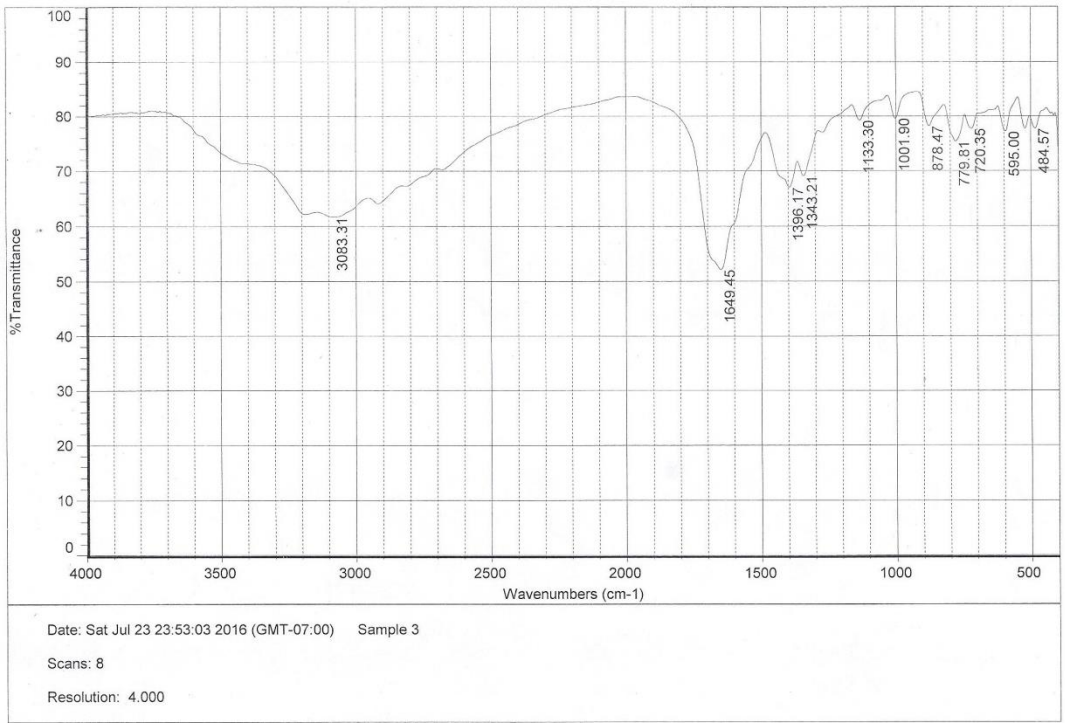


Figure 3 .45 Infrared Spectrum of Sample (3)

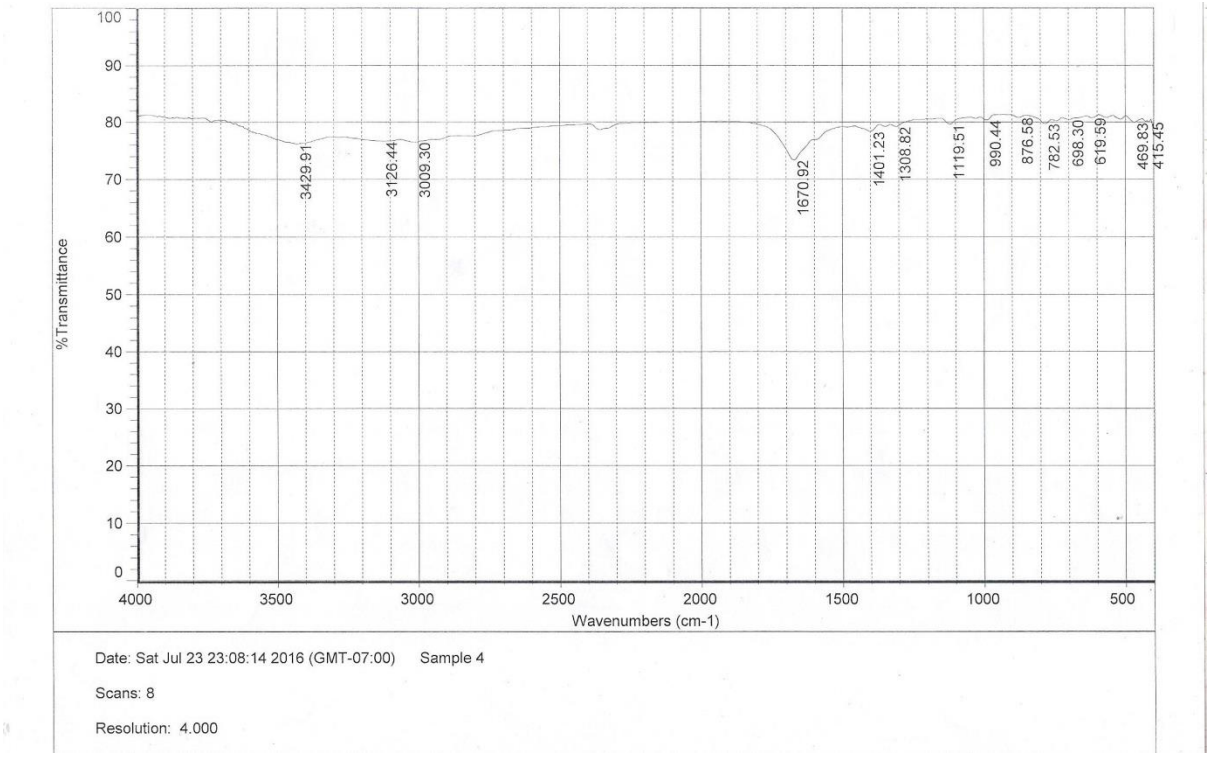


Figure 3.46 Infrared Spectrum of Sample (4)

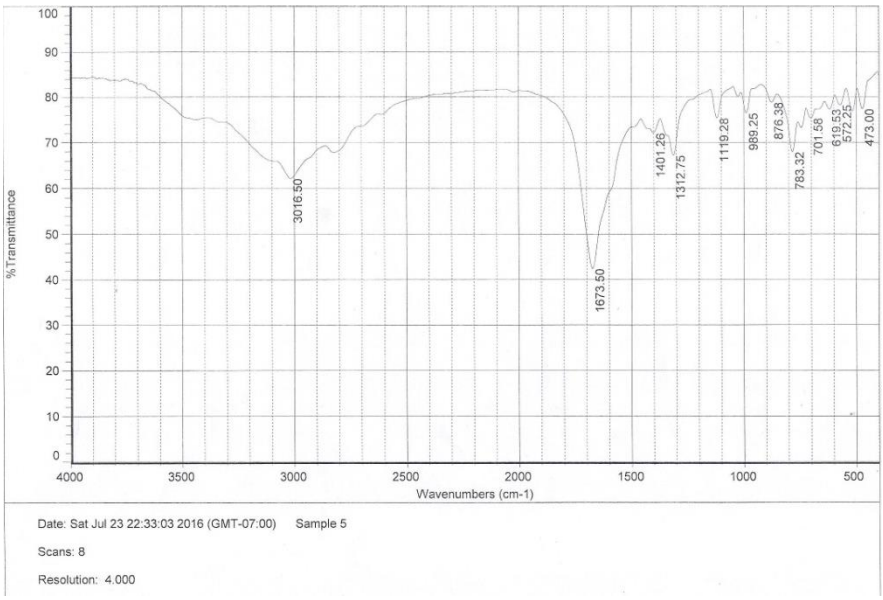


Figure 3.47 Infrared Spectrum of Sample (5)

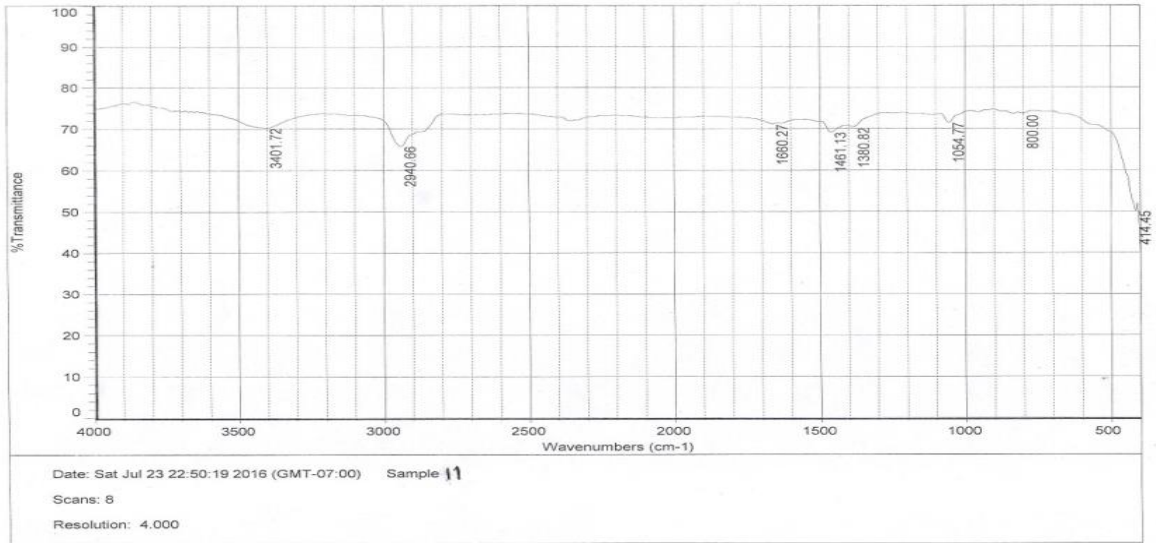


Figure 3.48 Infrared Spectrum of Sample (11)

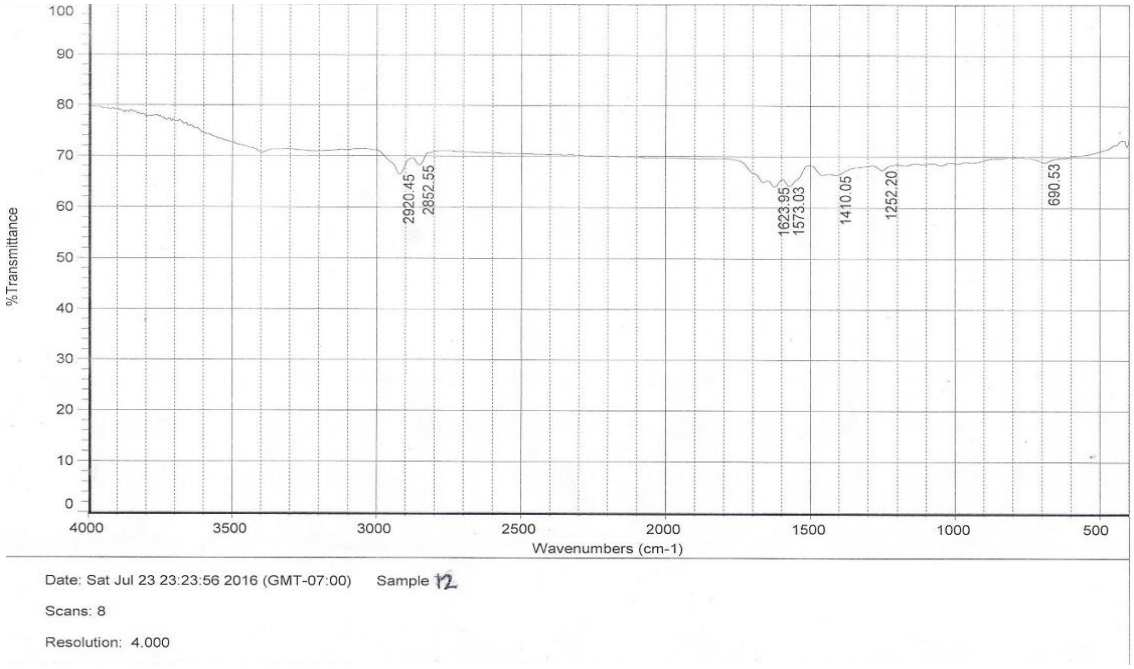


Figure 3.49 Infrared Spectrum of Sample (12)

Figure 3.50 Infrared Spectrum of Sample (13)

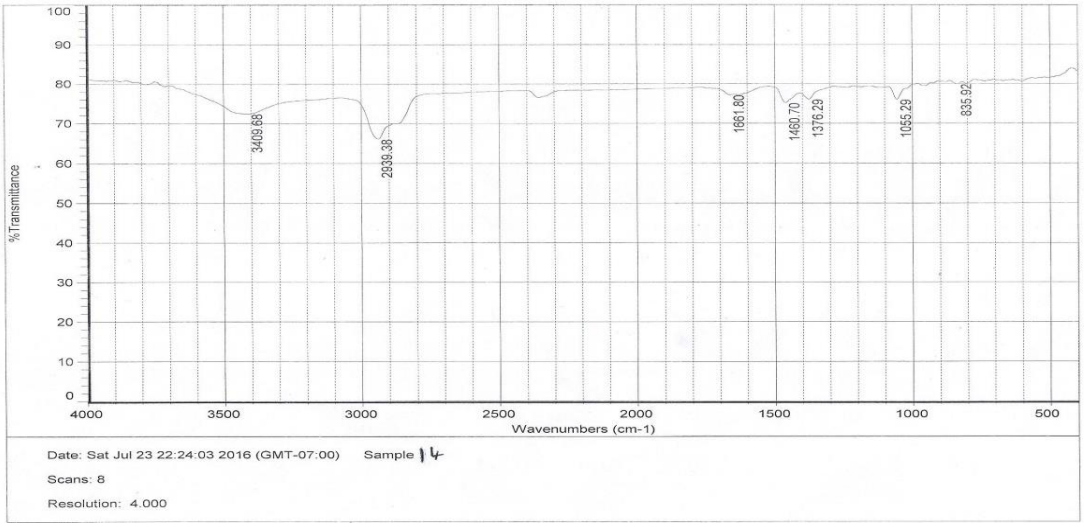


Figure 3.51 Infrared Spectrum of Sample (14)

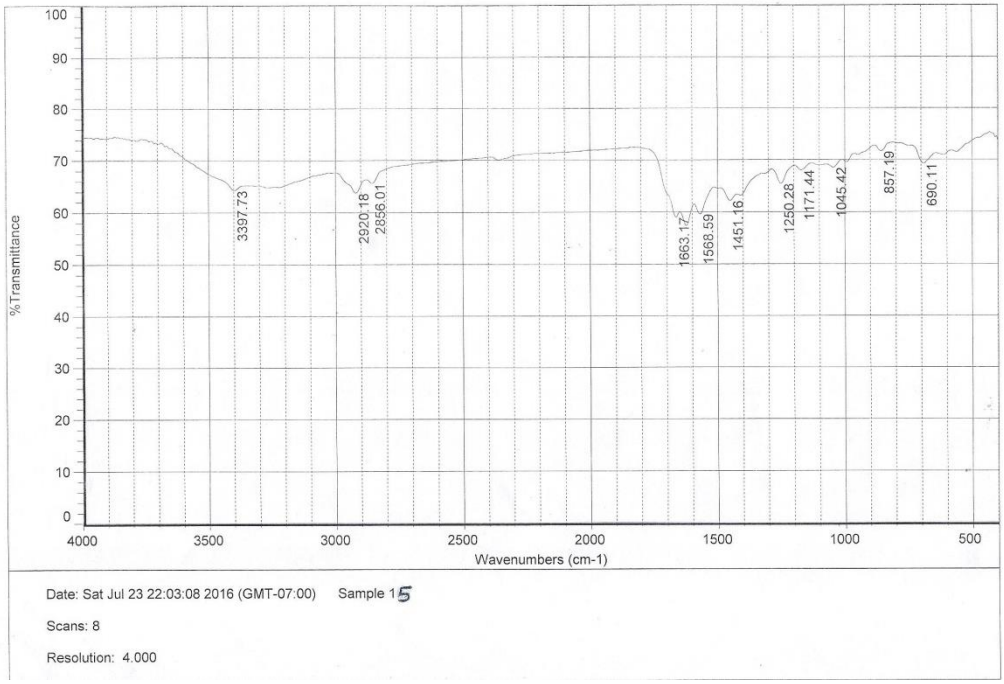


Figure 3.52 Infrared Spectrum of Sample (15)

IR-bands observed in standards and literature. Were compared with the IR spectra of 5 kidney stone samples (1-5) .It has been found that samples 1, 3, 4 and 5 were composed of calcium oxalate while Sample 2 was composed of uric acid. Found that calcium oxalate stones mainly develop due to hyperoxaluria, which is a metabolic disorder that causes the stone formation. Oxalate is end product of several metabolic pathways (including those involving serine, glycine, hydroproline and ascorbate). 10 to 20% urinary oxalate stones are derived from dietary sources and foods rich in oxalate such as cranberries, spinach, chocolate and tea. Any condition that increases oxalate absorption from food may lead to increased oxalate production and cause calcium oxalate stone formation (137).

IR-bands observed in standards and literature was compared with the IR spectra of 5 gall stone samples (11-15). It has been found that all samples were composed from mixed stones.

In sample number (1) the peak in region (784.26) near 778cm-1 due to (C=O) asymmetrical stretching, in region (1304.69) near 1314.39 cm-1 due to (C-C) a symmetrical stretching, and in region (1674.44) near1604.64 cm-1 due to (OC=O) asymmetrical stretching. This sample composed from calcium oxalate.

In sample number (2) the peak in region (1654.65) near 1637.29cm-1 due to (C=c) stretching, in region (1029.13) near 1018 cm-1 due to (N-H) stretching, and in region (758.67) near738.3cm-1 due to (C-N) stretching of aromatic . These samples composed from uric acid

In sample number (3) the peak in region (779.81) near 778cm-1 due to (C=O) asymmetrical stretching, in region (1343.21) near 1314.39 cm-1 due to (C-C) a symmetrical stretching, and in region (1649.45) near1604.64 cm-1 due to (OC=O) asymmetrical stretching. This sample composed from calcium oxalate.

In sample number (4) the peak in region (782.53) near 778cm-1 due to (C=O) asymmetrical stretching, in region (1308.82) near 1314.39 cm-1 due to (C-C) a symmetrical stretching, and in region (1670.92) near1604.64 cm-1 due to (OC=O) asymmetrical stretching. These samples composed from calcium oxalate.

In sample number (5) the peak in region (783.32) near 778cm-1 due to (C=O) asymmetrical stretching, in region (1312.75) near 1314.39 cm-1 due to (C-C) a symmetrical stretching, and in region (1673.50) near1604.64 cm-1 due to (OC=O) asymmetrical stretching. This sample composed from calcium oxalate

Table 3.3 Infrared IR-bands for Kidney stone samples (1-5)

Types of Stone	**IR-bands observed in samples**	**IR-bands observed in standards and literature(Naseem Aslam Channa2005)**
Calcium oxalate (pure)	**1. 784.26 ,1304.69, 1674.44** **3. 779.81,1343.21,1649.45** **4. 782. 53,1308.82,1670.92** **5. 783.32,1312.75,1673.50**	**778.53 (C=O asymmetrical stretching), 1314.93 (C-C symmetrical stretching), 1604.64 (OC=O asymmetrical stretching)**
Uric acid (pure)	**2. 1637.65,1029.13,758.67**	**1637.29 (C=C stretching),1018.13(N-H stretching),738.03 aromatic) (C-N stretching of**

In sample number (11) (3401.72) near 3410 due to CH2 and CH3 asymmetric stretching, in region (2940) near2925 cm–1 due to CH2 and CH3 asymmetric Stretching ,In region (1660.27) near 1670, 1640 due to (OC=O) stretching. In region(1461.13) cm–1 near 1460 due to CH2 and CH3 bending .In region (1380.82) cm–1 near 1380 due to CH2 and CH3 bending. In region (1054.77) near 1050cm–1 due to (C-C stretching). This samples composed from cholesterol and calcium biliurbinate (Mixed stones)

In sample number (12) in region (2940) near2925 cm–1 due to CH2 and CH3 asymmetric Stretching , In region (2852.55) near2860 due to(CH2 and CH3 symmetric stretching) In region (1623.95) near 1670, 1640 due to (OC=O) stretching. In region (1410.05) cm-1near 1460 due to CH2 and CH3 bending. In region (1252.20) near 1050cm–1 due to (C-C stretching). These samples composed from cholesterol and calcium biliurbinate. (Mixed stones)

In sample number (13) in region (3400.67) near 3410 due to CH2 and CH3 asymmetric stretching, in region (2938) near2925 cm–1 due to CH2 and CH3 asymmetric Stretching ,In region (1629.33) near 1670, 1640 due to (OC=O) stretching. In region(1460.83) cm–1 near 1460 due to CH2 and CH3 bending .In region (1374.29.) cm–1 near 1380 due to due to CH2 and CH3 asymmetric stretching. In region (1054.77) near 1050cm–1 due to (C-C stretching). This samples composed from cholesterol and calcium biliurbinate (Mixed stones)

In sample number (14) in region (3409.68) near 3410 cm–1 due to CH2 and CH3 asymmetric stretching, in region (2939.38) near2925 cm–1 due to CH2 and CH3 asymmetric Stretching , In region (1661.80) near 1670, 1640 due to (OC=O) stretching ,in region (1460.70) cm–1 near 1460. Due to CH2 and CH3 bending , In region (1376.29.) cm–1 near 1380 due to due to CH2 and CH3 asymmetric stretching. in region (1055.29) near 1050 cm–1 due to due to (C-C stretching). These samples composed from cholesterol and calcium biliurbinate. (mixed stones).

In sample numbers (15) in region (3397.73) near 3410 due to CH2 and CH3 asymmetric stretching, in region (2940) near2925 cm–1 due to CH2 and CH3 asymmetric Stretching , In region (2856.01) near2860 due to(CH2 and CH3 symmetric stretching). In region (1663.17) near 1670, 1640 due to (OC=O) stretching. . In region(1568.59) cm–1 near 1575 due to (C=C stretching) ,In region(1451.16) cm–1 near 1460 due to CH2 and CH3 bending .In region (1250.28) cm–1 near 1380 due to CH2 and CH3 bending. In region (1054.77) near 1050cm–1 due to (C-C stretching). These samples composed from cholesterol and calcium biliurbinates. (Mixed stones)

Table 3.3 IR bands of principal components observed in gallstones

Types of Stone	IR-bands observed in samples	IR-bands observed in standards and literature (Reference [Zhou X-S 1997])
Pure cholesterol		2925 (CH2 and CH3 asymmetric stretching), 2860 (CH2 and CH3 symmetric stretching) 1460, (CH2 and CH3 bending), 1050 (C-C stretching)
Cholesterol+bilirubin	11. 3401.72,2940.66,1660.27, 1461.13 ,1380.82,1,1054.77 12.2920.45,2852.55,1623.95, 1573.03,1410.05, 1252.20,690.53 13.3400.67,2938.00,1629.33,1460, 1374.29,1054.56 14.2939.38,1661.80,1460.7,1376.29, 1055.29 15.3397.73, 2920.18, 2856.01, 1663.17, 1568.59 ,1451.16 ,1250.28, 1171.44 ,1045.42 , 857.19 , 690.11	Same as in pure cholesterol and 1670, 1640 (OC=O stretching), 1575 (C=C stretching)
Calcium bilirubinate		3410 (CH2 and CH3 asymmetric stretching), 1380 (CH2 and CH3 bending)

4. Conclusion

Based on above results it may be conclude that the dietary habits, genetic factors, metabolic conditions, and lifestyle play an important role in the formation of stones. Hence a simple and important lifestyle change to prevent stones is to drink more liquids –water is best (at least 8-10glasses of water day).Normal –Calcium, low Sodium, and low animal protein diets are recommended for stone prevention.

Males are commonly to Kidney stone formation than females while females are commonly to gallstone formation than males.

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