An-Najah National University

Faculty of Graduate Studies

Preparation of Sulfur Nanoparticles and Investigating their Activities Against Cancer Cells

By

Anas Khaled Abed Al Ali

Supervisor

Dr. Mohammed Suleiman

Co-Supervisor

Dr. Ayman Hussein

This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master in chemistry, Faculty of Graduate Studies, An- Najah National University, Nablus, Palestine.

2013

Preparation of Sulfur Nanoparticles and Investigating their Activities Against Cancer Cells

By

Anas Khaled Abed Al Ali

This Thesis was defended successfully on 5 /12 /2013 and approved by:

Defense Committee Members	**Signature**
1. Dr. Mohammed Suleiman / Supervisor	.....
2. Dr. Ayman Hussein / Co-Supervisor	.....
3. Dr. Hatim Salim / External Examiner	Hatim Salim
4. Prof. Dr. Ismail Warad / Internal Examiner	.....
5. Dr. Mohyeddin Assali / Internal Examiner	.....

Dedication

SUBMITTED WITH GRATEFUL RECOGNITION & APPRECIATION TO MY GREAT MOTHER & MY WONDERFUL FATHER.

TO MY BROTHERS, SISTERS & THEIR FAMILIES.

TO ALL TEACHERS IN MY ENTIRE LIFE.

TO THE MEMORY OF MY DEAREST FRIENDS.

I DEDICATE THIS WORK.

Acknowledgement

After thanking Allah, who granted me the ability to finish this work, I would like to express my sincere gratitude to my thesis supervisors: Dr. Mohammed Suleiman for all his guidance, understanding, support and sound advice in all aspects of my research work and Dr. Ayman Hussein for his scientific support, encouragement, guidance and constructive advice.

Also my sincere thanks go to my doctors Prof. Dr. Hikmat Hilal, Dr. Waheed Jundi, Dr. Shehdeh Jodeh, Dr. Nidal Zatar, Dr. Samar Al-Shakshir and Prof. Dr. Mohammed Al-Nuri for their help and support during my study.

I also thank the thesis committee member, Dr. Hatim Salim, Dr. Mohyeddin Assali and Prof. Dr. Ismail Warad for their consent to read my thesis and provide useful suggestions.

Thanks to Hamdi Mango Center for Scientific Research at the University of Jordan and the unity of the electron microscope at Yarmouk University for their collaboration in SEM and TEM measurements.

I appreciate help and support from all members of the Chemistry Department at An-Najah National University especially laboratory technicians, in particular Mr. Omair Nabulsi and Mr. Nafiz Dweikat.

My colleagues and friends in the Departments of Chemistry deserve warm thanks, for the assistance and support during the period of my study and to spend the most beautiful fun times that we spent together.

Also my sincere thanks to Dr. Yazid Al-Jayyousi and Abdullah Hussein for his help, efforts, motivation and support during my study.

Thanks to my family with all my love, especially my Mother and Father, who stood with me throughout my study and provided me with psychological support and encouragement.

Anas Al Ali

الإقرار

أنا الموقع أدناه موقع الرسالة التي تحمل العنوان:

Preparation of Sulfur Nanoparticles and Investigating their Activities Against Cancer Cells

تحضير حبيبات نانومترية من الكبريت وبحث فعاليتها ضد الخلايا السرطانية

أقر بأن ما اشتملت عليه الرسالة هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة أو لقب علمي أو بحثي لدى أي مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provide in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ:**

Table of Contents

List of Tables

List of Figures

List of Abbreviation

NPs	Nanoparticles
S NPs	Sulfur Nanoparticles
SDBS	Sodium dodecyl benzene sulfonate
SDS	Sodium dodecyl sulfate
PNPs	Polymeric nanoparticles
CTAB	Cetyl trimethyl ammonium bromide
S-TOAB	Sulfur stabilized with Tetraoctylammonium bromide
QACs	Quaternary ammonium compounds
TOAB	Tetra octylammonium bromide
ROS	Reactive oxygen species
TBARS	Thiobarbituric acid reactive substances
GSH	Glutathione
MDA-MB-231	Human breast adenocarcinoma
C6 glioma	Human brain cancer
Bcl-2	B-cell lymphoma 2
HEK293	Human Embryonic Kidney 293 cells
HT-29	Human colon adenocarcinoma grade II cell line
XRD	X-Ray Diffraction
SEM	Scanning electron microscope
TEM	Transmission electron microscope
DMEM	Dulbecco's Modified Eagle Medium

Preparation of Sulfur Nanoparticles and Investigating their Activities Against Cancer Cells

By
Anas Khaled Abed Al Ali
Supervisors
Dr. Mohammed Suleiman
Dr. Ayman Hussein

Abstract

Sulfur nanoparticles (S NPs) were prepared by a quick precipitation method in the absence and presence of tetraoctylammonium bromide (TOAB) that was used as a stabilizer to control the nanoparticles size. Scanning electron microscope (SEM), transmission electron microscope (TEM) and X-Ray Diffraction (XRD) were used to characterize S NPs.

The average size of S NPs was in the range 2.4 – 8.9 nm, with spherical like shape. The size was controlled the changing the preparation parameters: temperatures, different acid concentration, different acid type and the presence of TOAB stabilizer.

The anticancer activity of the prepared S NPs has been tested on various types of cancer cell clones including leukemia, kidney and colon cancers. As a control, the activity of the prepared S NPs was investigated on normal human cell line (Lax cells).

The prepared S NPs were found to have selective toxicity to the three cancer cell lines investigated in this study.

For example at concentration of 50µg/mL, 20 and 100% effect on colon and kidney cancer cells, respectively. However, at 100 µg/mL concentration of S NPs showed 15% toxicity of leukemia cancer cells.

The same S NPs at concentration of 10 µg/mL lead to 85% killing of kidney cancer cell.

At a concentration of 100 µg/mL of S NPs, no toxicity effect on normal human cells (Lax cell line) was found. Additionally, toxicity of S NPs was found to have a time-dependent toxicity pattern.

In this work, the toxicity of S NPs was found to be at least 100 times more effective than other reported results of SiO_2 and amorphous chitin NPs preparations. The high potency in selective killing of cancer cells by the prepared S NPs recommends further investigations.

Chapter 1
Introduction

1.1 Introduction to nanotechnology

Nanoparticles (NPs) are typically in the size range of 1–100 nm, and can have different shapes and compositions from bulk counterpart. Nanoparticles usually have different and more interest physical and chemical characteristics from those of the same material in bulk form [1].

There are various applications of the NPs such as in medical applications including anticancer and antibacterial activity [2-3], fuel cell [4], (solid oxide, hydrogen storage) [5], catalysis [6-7], semiconductors and water treatment [8-9].

1.1.1 Types of nanoparticles

Nanoparticles can be categorized into groups, like inorganic (metallic and nonmetallic) nanoparticles [10], polymeric nanoparticles [11], solid lipid nanoparticles [12], liposome's, with different shapes and structures such as; crystalline [13], nanocrystal [14], spherical NPs [15], nanotubes [16], nanorods and dendrimers [17].

Polymeric nanoparticles (PNPs) are biodegradable and biocompatible, and adopted as a preferred method for nanomaterial drug delivery. The advantages of using (PNPs) in drug delivery are most important that they generally increase the stability of any volatile pharmaceutical agent, also polymeric nanoparticles have been engineered specificity, to allowed the

delivery of higher concentration of the pharmaceutical agent to the desired location [18].

Nanocrystal are aggregates of molecules that can be combined into a crystalline form of the drug surrounded by a thin coating of surfactant, they are used in materials research, chemical engineering and quantum dots for biological imaging [19].

Nanotubes are self-assembling sheets of atoms arranged in tubes and may be organic or inorganic in composition and can be produced as single- or multi-walled structures. Nanotubes have large internal volumes and the external surface area can be easily functionalized [20].

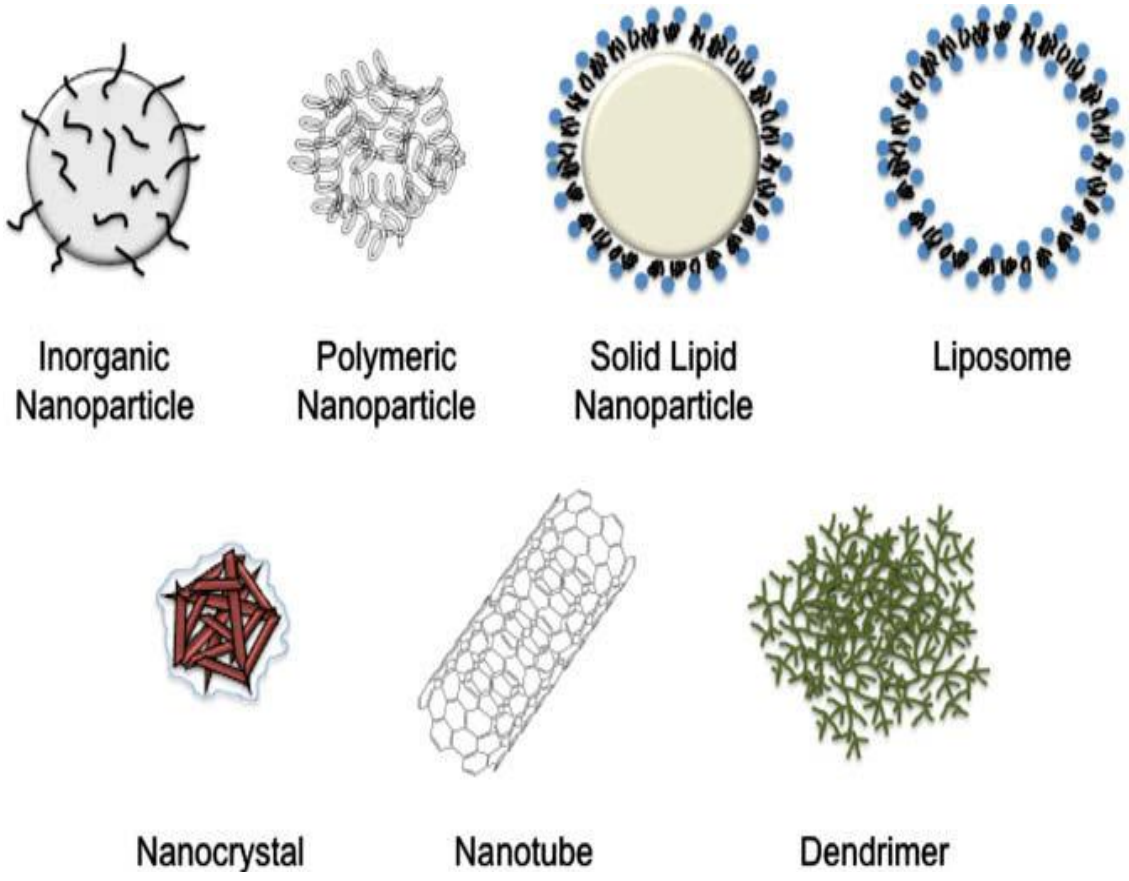

Fig 1.1: Various types of nanoparticles [20]

1.1.2 Nanoparticles preparation methods

Metallic and nonmetallic nanoparticles can be prepared by different chemical and physical methods. The chemical methods such as: chemical vapor deposition and Wet Chemical methods; salt reduction [21], electrochemical techniques [22], Thermal decomposition techniques [23-25], and microemulsion technique [26]. Physical methods such as condensation and laser ablation [27].

To prevent undesirable agglomeration of the nanoparticles during synthesis processes, stabilizing technique is usually used in which a protecting shell (surfactant) is used, such as polymer, solid matrix and surfactants [28]. Surfactants have many practical activities in chemical and medical applications and can influence the size, the shape, the structure, and the efficiency of the nanoparticles in various applications [29].

Wet chemical method is the most common process for nanoparticles synthesis, since it’s nonexpensive, quick and produce large yield. In the technique, metal salts are dissolved in a solvent such as alcohol or water, salts then dissociate to metal cations and nonmetal anions. The metal cations are reduced to zero-valent metal which then aggregates to form nanoparticles by nucleation and growth steps. Finally nanoparticles form a precipitate or are forced to be precipitate. The size of the nanoparticles can be controlled by varying the pH, temperature, solvent types and reducing agents type [30-32].

Electrochemical technique is used widely to prepare metallic nanoparticles, This method can be summarized in several steps; The first step is oxidative dissolution of anode by applying current density on it, migration of the cations into the cathodes, reduction of cations at the cathode to zero-valent state, agglomeration to form nanoparticles by nucleation and growth, arrest of growth by stabilizers to prevent the nanoparticles from non-desired aggregation, and finally precipitation of the produced nanoparticles. The size of the nanoparticles can be simply controlled by varying of temperature, solvent and current density [33].

Thermal decomposition is another simple and fast technique to prepare NPs, for example Palladium NPs were prepared and stabilized in TOAB by using the electrochemical method [34]. The Pd NPs have different sizes between 3.0 and 6.0 nm which were prepared selectively by setting the preparation parameters [35-36].

Cadmium oxide (CdO) NPs which were prepared starting from organometallic complex through one step calcinations process at 800 °C. The obtained CdO NPs have a size of about 50 nm [37].

Gold nanospheres (also known as gold colloids) of 2nm to over 100 nm in diameter were synthesized by controlled reduction of an aqueous $HAuCl_4$ solution using different reducing agents under varying conditions [38].

1.1.3 Sulfur

The sulfur, as gores-grain (bulk), micro and to much less extent as nano, is widely used in different industrial applications activities such as production of sulfuric acid, nitrogenous fertilizers, enamels, antimicrobial agents, gun powders, phosphatic fertilizers, plastics, petroleum refining, pulp and paper industries, other petrochemicals, ore leaching processes and different other agrochemical industries [39] .

Sulfur can also be used in agricultural applications and used against many fungal plant diseases such as apple scab disease in the cold conditions, in conventional culture of grapes, vegetables, strawberry and many cultivated plants. Sulfur can be regarded as high-efficiency of pesticides used in agriculture, where it has a good effect against a wide range of powdery mildew disease, as well as black spots that infect plants [31].

1.1.4 Sulfur nanoparticles applications

Sulfur nanoparticles (S NPs) have many applications now adays such as; modification of metal and carbon nanotubes, and synthesis of nanocomposites for lithium batteries [40], anti-cancer agent [41-42], antibacterial agent [43], agrochemical industries [39] fungicides in agriculture fields [44], synthesis of sulfur nanowires with carbon to form hybrid materials with useful properties for gas sensor and catalytic applications [45] and as adsorbent for the extraction of metal ions [46].

1.1.5 Sulfur nanoparticles preparation methods

Different methods were used to prepare nanosized sulfur nanoparticles [47], one of the methods used for synthesis of S NPs is wet chemical precipitation method by dissolving the sodium thiosulfate in double distilled water and different acidic solutions, using different surfactants (TX-100, CTAB, SDBS, and SDS) as stabilizer to control the particle size. The anionic stabilizer SDBS was found to be higher effective for obtaining a uniform sizes NPs. While, the smallest size (30 nm) S NPs was obtained by using CTAB as stabilizer [31].

Monoclinic S NPs have been prepared via the chemical reaction between sodium polysulfide and hydrochloric acid in a reverse microemulsions system, with theoline, butanol and a mixture of Span 80 and Tween 80 (weight ratio 8:1) as the oil phase, surfactant and co-surfactant, respectively. Transparent microemulsions were obtained by mixing the oil phase; a surfactant, co-surfactant, and the aqueous phase in appropriate proportion using an emulsification machine at room temperature. The S NPs prepared via this method were found to have an average diameter of around 20 nm, with a narrow size distribution, uniform spherical shape, and high purity [48].

S-NPs were synthesized from hazardous H_2S gas using novel biodegradable iron chelates in water/organic microemulsion system [47]. Ferric malic acid chelate was studied in water/organic microemulsion containing cyclohexane, n-hexanol and Triton X-100 as oil phase, a surfactant, co-surfactant, respectively, for the catalytic oxidation of H_2S

gas at ambient conditions of temperature, pressure, and neutral pH. The S NPs was nearly uniform in size (average particle size 10 nm) with narrow particle size distribution (in range of 5–15 nm) as compared to that in aqueous surfactant systems [49].

An electrochemical method is used to prepare the sulfur nanoparticles from thiosulfate ion. The size of S NPs obtained was in the range of 35 and 65 nm by adjusting the operation parameters including; the initial concentration of sodium thiosulfate. It was found that, the use of hot alcohol and cold water as solvent/non-solvent system along with 100 mL .min^{-1} flow rate for co-mixing of non-solvent resulted in the formation of S NPs in a typical size of 250 nm that are fairly homogeneous in shape and have a narrow particle size distribution [50].

1.2 Quaternary ammonium cations as disinfectant

Tetraoctylammonium bromide (TOAB) is one of the quaternary ammonium compounds with the molecular formula $[CH_3(CH_2)_7]_4N(Br)$ and molecular weight 546.79 g/mol. Quaternary ammonium compounds (QACs) belong to N-containing organic cations. The general formula is R_4N^+ where N has a positive charge. Nitrogen is bonded to four carbon atoms by covalent bonds; R is a saturated or unsaturated alkyl or aryl group. Wide range of quaternary ammonium cations are produced synthetically. It has various applications such as cosmetics, asphalt emulsions and as antimicrobials [51-52].

1.3 Cancer

Cancer is defined as uncontrolled cell growth. There are over 100 different types of malignancies, and each is classified by the type of cell that is initially affected. The cancer is one of the leading causes of death in the modern world, with more than 10 million new cases each year, and more than 5 million deaths annually [53].

Cancer can be diagnosed by a number of methods, including the presence of specified signs and symptoms, medical imagine or screening tests such as biopsy or molecular techniques. Cancer is usually treated with chemotherapy, radiation therapy and surgery [54].

Cancer is a multi factorial disease which means that there are several risk factors that are associated with it including environmental and genetic (family history) factors. Common environmental factors that contribute to cancer death include tobacco (25–30%), diet and obesity (30-35%), infections (15–20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity, and environmental pollutants [55].

1.3.1 Origins of Cancer

All types of cancer begin in the cells, the human body is made up of several types of cells. These cells grow and divide in a controlled manner to produce more cells as they are necessary to keep the body healthy. When cells become damaged or old, they will die and are replaced with new cells.

However, sometimes this orderly process goes wrong. The genetic material (DNA) of the cell can become damaged or changed, producing mutations that affect the growth and division of cells. When this happens, the cells grow in an uncontrolled manner resulting in the appearance of tumors which can be benign or metastasis. Benign tumors are not cancerous, but often can be removed, and in most cases they do not come back.

Malignant tumors are cancerous. Cells in these tumors can invade nearby tissues and spread to other parts of the body. The spread of cancer from one part of the body to another is called metastases. Some types of cancers do not form tumors. For example, leukemia is a cancer of the bone marrow and blood [56].

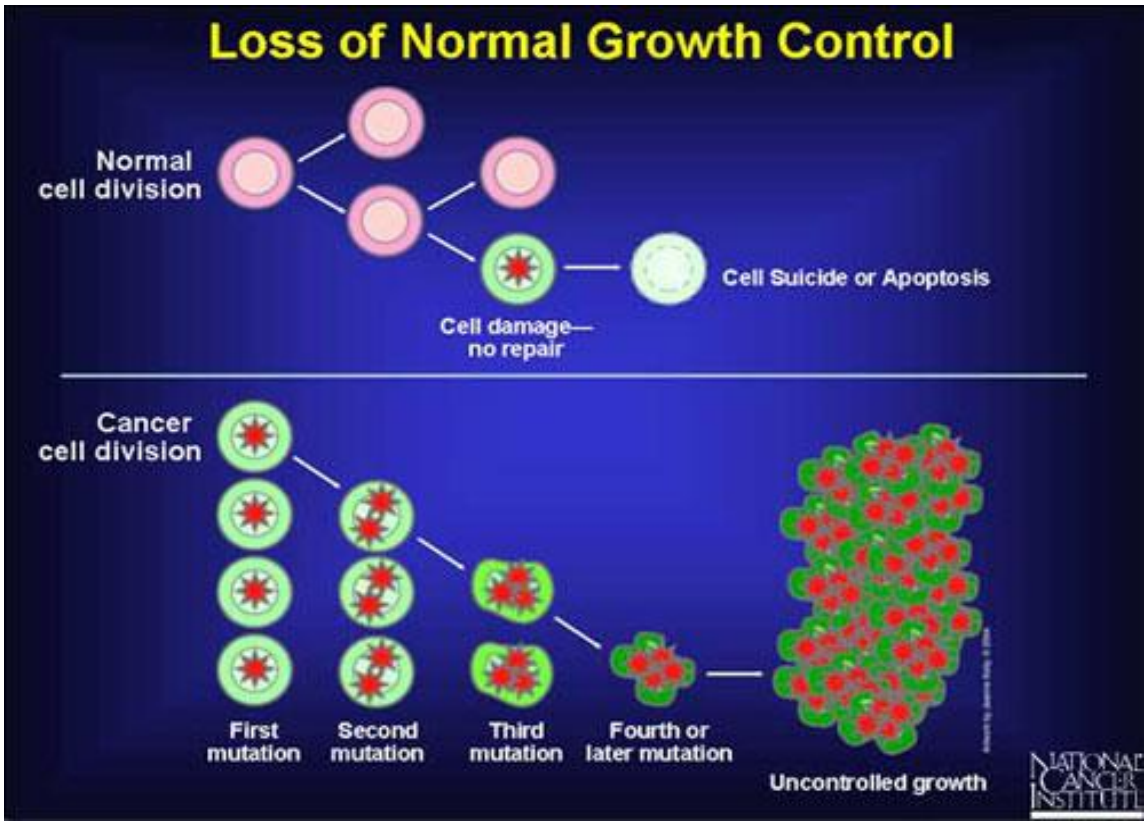

Fig 1.2: loss of normal growth control [56].

1.3.2 Cancer types

Types of cancer can be classified into broad categories. The main categories of cancer include:

- **Carcinoma:** cancer that begins in the skin or in tissues that line or cover internal organs. There are a number of sub-types of carcinoma, including adenocarcinoma, basal cell carcinoma, squamous cell carcinoma and transitional cell carcinoma.
- **Sarcoma**: a cancer that begins in bone, muscle, cartilage, fat, blood vessels, or other connective or supportive tissue.
- **Myeloma and lymphoma**: cancers that begin in the cells of the immune system.
- **Leukemia**: cancer that begin in blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood.
- **Central nervous system cancers**: cancers that begin in the tissues of the spinal cord and brain [56].

1.3.3 Cancer nanotechnology

Cancer nanotechnology is emerging as a new field of interdisciplinary research fields, cut across the disciplines of biology, chemistry, medicine, engineering, and is expected to lead to significant progress in cancer detection, diagnosis, and treatment.

The basic rationale is that metals, polymeric particles and semiconductors have novel optical, magnetic, electronic and structural

properties which are often not available of individual molecules or bulk solids.

Medical applications also appeared, such as the use of super paramagnetic iron oxide NPs as a contrast agent for lymph node prostate cancer detection and the use of polymeric NPs for targeted gene delivery to tumor vasculatures. New technologies using metal and semiconductor NPs are also under intense development for molecular profiling studies and multiplexed biological assays [57].

1.3.4 Leukemia cancer

Leukemia is a type of cancer of the blood or bone marrow characterized by an abnormal increase of immature white blood cells called blasts. In turn, is part of a wider group of diseases that affect the blood, bone marrow and lymphatic system, which are all known as hematological neoplasm's [58]. There are four major kinds of leukemia are shown in Table 1.1.

Table 1.1: Four major's kinds of leukemia cancer [59].

Cell type	Acute	Chronic
Lymphocytic leukemia (or "lymphoblastic")	Acute lymphoblastic leukemia (ALL)	Chronic lymphocytic leukemia (CLL)
Myelogenous leukemia (also "myeloid" or "nonlymphocytic")	Acute myelogenous leukemia (AML) (or myeloblastic)	Chronic myelogenous leukemia (CML)

Experts do not know what causes leukemia. But it is known that some things increase the risk of some types of leukemia. These things are called risk factors.

The most likely susceptible to leukemia in the following cases when exposed to a large amount of radiation, exposure to certain chemicals at work such as benzene, some types of chemotherapy for the treatment of cancer, another is to have Down's syndrome or some genetic problems and smoke.

There are some common symptoms of the disease such as: fever, night sweats, headaches, joint pain or bone, a swollen or painful belly from an enlarged spleen, feeling very tired or weak, losing weight and not feel hungry lymph nodes and swollen lymph in the armpit, neck or groin [60].

There are many methods used to treat leukemia, the most widely used treatments on a wide range of chemotherapy and radiation therapy in some cases, or bone marrow transplant [58].

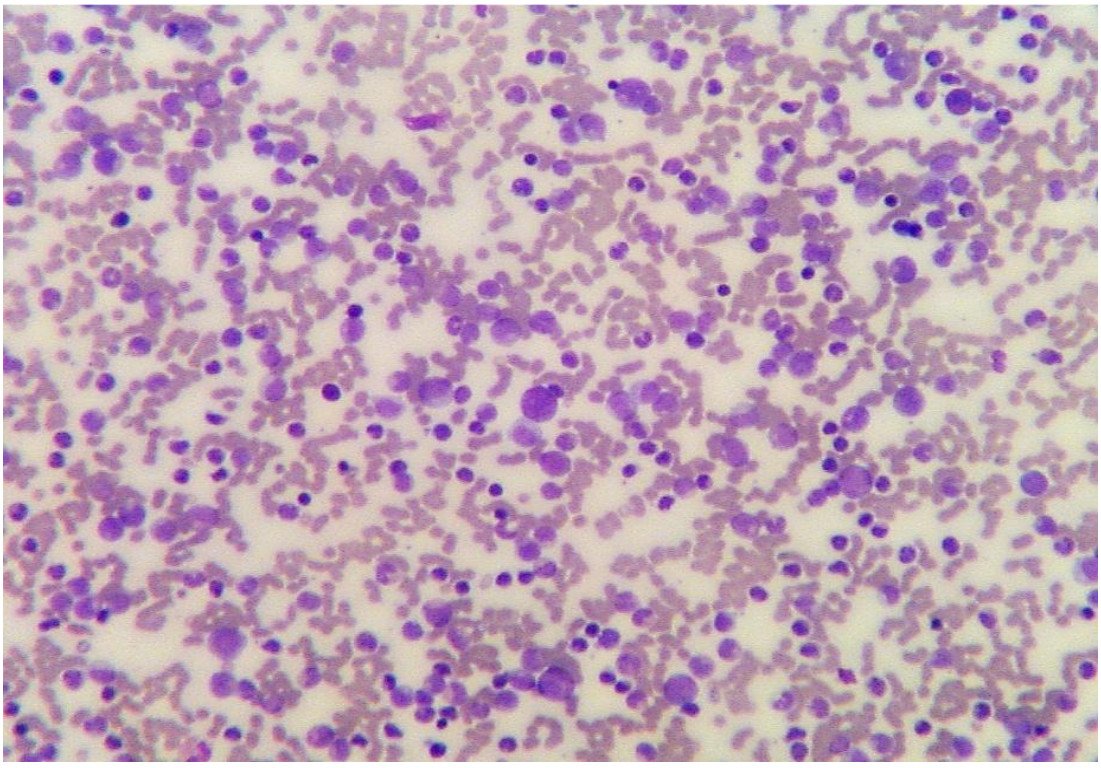

Fig 1.3: leukemia cancer [WBCs (blue cells) are extremely numerous RBCs are much less numerous than in normal blood] [61].

1.3.5 Kidney cancer

Kidney cancer is a type of cancer that forms in tissues of the kidney. Kidney cancer generally spreads to other parts of the body at a slower pace than other cancers. There are two main types of this cancer classified by histological origin are renal cell carcinoma (RCC) and transitional cell carcinoma (TCC), It also includes Wilms tumor, which is a type of kidney cancer that usually develops in children under the age of 5 years [62] .

Renal cell carcinoma (RCC) is the kind that arises from renal cell carcinoma and be only in the kidney, and accounts for 80-85% of kidney tumors. Transitional cell carcinoma (TCC) is the kind that arises from the transitional cells and can affect the kidney, renal pelvis, ureter, bladder or urethra and constitutes approximately 10% of kidney tumors.

The exact causes of kidney cancer is not specified up to now, but its believed that the following factors may give rise to the development of kidney cancer. These factors are smoking, dialysis for a long time, obesity and high blood pressure, as continuous exposure to chemicals [63].
Signs and symptoms most commonly used in kidney cancer is a lump in the kidney, blood in the urine, painful spasms in the bladder, mild pain in the side, fatigue and weight loss.

The methods used for the treatment of kidney cancer are surgery, chemotherapy, radiation therapy, immunotherapy, and vaccine therapy [64].

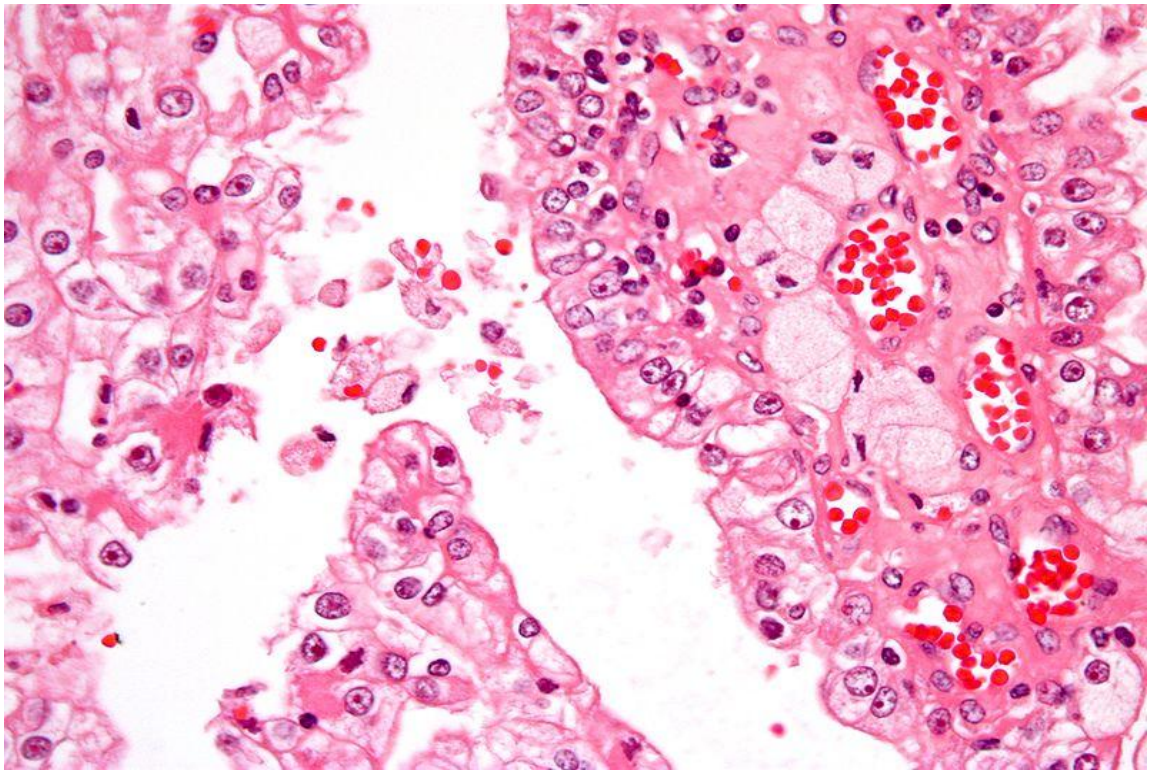

Fig 1.4: kidney cancer [renal cell carcinoma] [65].

1.3.6 Colon cancer

Colon cancer is a type of cancer that forms in tissues of the colon. Colon cancer is the third type of cancer in the world and affects the elderly, as it is the third cause of death from cancer.

In most people, colorectal cancers develop slowly over a period of several years. Before the development of cancer, the growth of tissue or tumor usually begins as a non-cancerous polyp on the inner lining of the colon or Rectum.

The tumor tissue is abnormal and can be benign (not cancer) or malignant (Cancer). A polyp is benign, non-cancerous tumor. Some polyps change in cancer, but not all:

1- Adenomatous polyps or adenomas:

Polyps that can change into cancer. Because of this, tumors are called pre-cancerous condition.

2- Inflammatory polyps and Hyperplastic polyps.

In general, are not pre-cancerous. But some hyperplastic polyps can become pre-cancerous, or it may be a sign of a greater risk of developing tumors and cancer, especially when these polyps grow in the ascending colon [66]. Most people who develop colon cancer does not appear to have any symptoms in the early stages of the disease.

When the symptoms of colon cancer begin to appear, they vary from one situation to another, and be linked to the size and location of the tumor within the colon.

The symptoms of colon cancer are blood in the stool, worsening constipation, decrease in stool calibre, loss of weight, loss of appetite and nausea or vomiting [67].

Risk factors that lead to colon cancer are older age, male gender, high intake of fat, red meat or alcohol, smoking, obesity and a lack of physical exercise [68].

The methods used for the treatment of colon cancer are surgery, chemotherapy, radiation therapy [69].

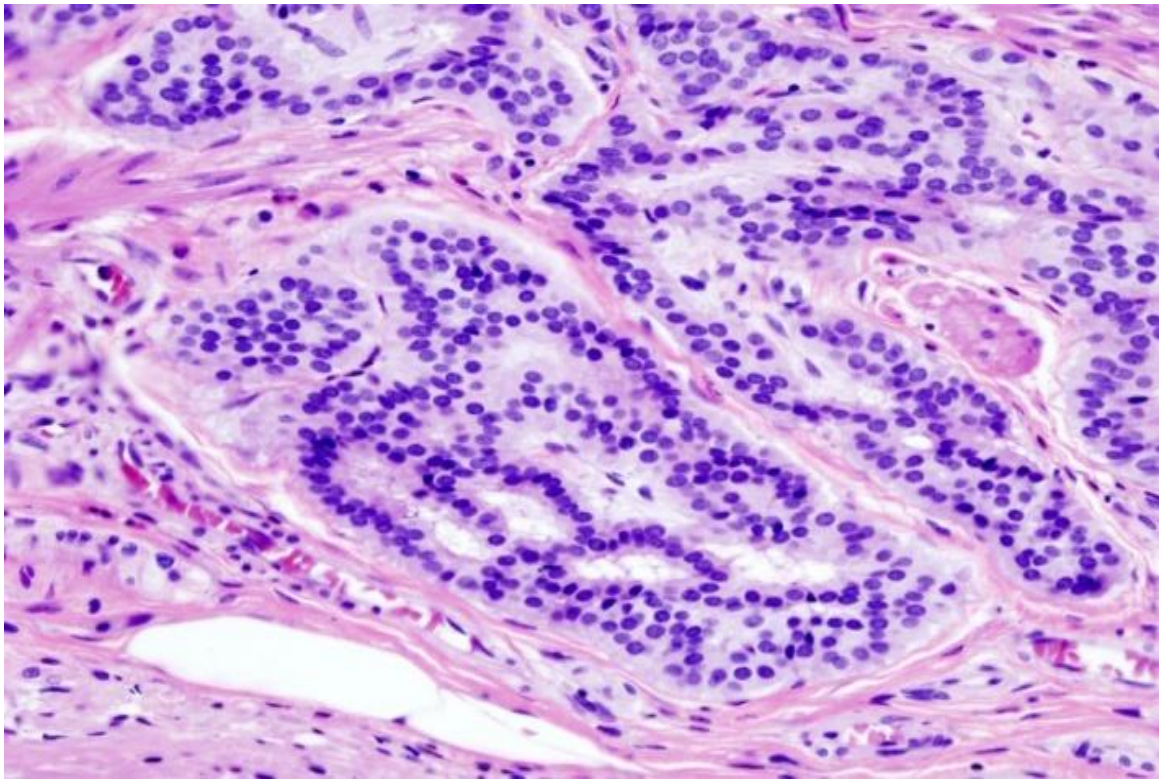

Fig 1.5: Colon cancer [70]

1.4 Literature review

F. Wanga et al. studied the cytotoxicity of two sizes of SiO_2 nanoparticles (20 and 50 nm) to investigate in cultured human embryonic kidney cells. It was found that exposure to SiO_2 nanoparticles at dosage levels of 20–100 µg/mL lead to cellular morphological modifications, mitochondrial dysfunction, and oxidative stress as indicated by elevation of intracellular ROS (reactive oxygen species) and TBARS (thiobarbituric acid reactive) substances, as well as depletion of GSH (glutathione), which triggers cell cycle arrest and apoptosis in a dose dependent manner [71].

S. Gurunathan et al. have investigated the cytotoxic effect of silver nanoparticles on breast cancer. An average range of diameter of Ag NPs

on MDA-MB-231(human breast adenocarcinoma) was 20 nm. Ag NPs have anti proliferative activity through induction of apoptosis in MDA-MB-231 breast cancer cell line, suggesting that biologically synthesized Ag NPs might be a potential alternative agent for human breast cancer therapy. This study showed the possibility of using Ag NPs to inhibit the growth of the tumor cells and their cytotoxicity for potential therapeutic treatments [72].

Y.An et al. using sulfur nanoparticles an average diameter of approximately 80 nm to inhibit C6 glioma (human brain cancer) cell proliferation and induced apoptosis in a dose and time dependent manner. Sulfur NPs significantly inhibited C6 glioma cell proliferation and promoted cell apoptosis by inducing the up regulation of Bax and down regulation of Bcl-2 (B-cell lymphoma 2) expression [41].

Nanotechnology has an emerging and promising field that uses NPs to facilitate the diagnosis and treatment of cancer. Nanoparticles have the potential to offer solutions to the current obstacles in cancer therapies, because of their unique size and large surface-to-volume ratios [73].

With advances in nanotechnology, and the understanding of properties of materials at the nano scale level, several distinct therapeutic systems have been approved or entered clinical development for several disease therapy [74].

1.5 Objectives of this study

In this research, the main objective is treatment of different cancer cells by using sulfur nanoparticles. Further specific objectives include:

1- Size selective synthesis of S NPs with and without TOAB surfactant matrix.

2- Characterization of S NPs with and without TOAB surfactant using SEM, TEM and XRD to determine the nanoparticles shape and size.

3- Studying the activity of the sulfur nanoparticles against cancer cells.

4- Studying the anticancer activity of many parameters including NPs size, stabilization, concentrations and contact time.

Chapter 2

Materials and Methods

2.1 Materials

Sodium thiosulfate ($Na_2S_2O_3$.$5H_2O$) (catalog #2355537600) was purchased from Frutarom Co., hydrochloric acid (HCl) (catalog # 100319) 32% conc. was purchased from Merck Co., nitric acid (HNO_3) 65% conc. (catalog # 30713) was purchased from Riedel Co., sulfuric acid (H_2SO_4) 98% conc. (catalog #19550201) was purchased from BiolabCo., phosphoric acid (H_3PO_4) 85% conc. (catalog # 2355527800) was purchased from Frutarom Co., tetraoctyl ammonium bromide (TOAB) 98% conc. (catalog # 294316-25G) was purchased from Sigma Co., Dulbecco's Minimal Essential Media (DMEM) (catalog # 01-055-1A) from was purchased from Biological Industries Co.

2.2 Sulfur nanoparticles preparation

Quick precipitation method was used to prepare two types of sulfur nanoparticles with and without tetraoctylammonium bromide (TOAB) surfactant as in the following reaction.

$$Na_2S_2O_3.5H_2O + \text{acidic sol'n} \xrightarrow[2)\,Heating]{1)\,TOAB\ Stabilizer} S^0_{n\,(cluster)}$$

2.2.1 Preparation of sulfur nanoparticles with TOAB surfactant

10.00 g of $Na_2S_2O_3$.$5H_2O$ dissolved in 50 mL of distilled water then transferred into 250 mL conical flask. 0.20 g of TOAB surfactant was dissolved in 20 mL of distilled water by heating to 65°C. A mixture of Sodium thiosulfate and TOAB was prepared by combining both solutions together and stirred mechanically at 120 rpm and heated in constant bath at 30, 40, 50 and 60 °C, respectively. Then 40 mL of different acid solutions were added to the mixture to produce different sized NPs under continuous stirring. After the reaction was stopped after 40 min, the produced yellow precipitates were collected, washed with distilled water and then dried.

2.2.2 Preparation of sulfur nanoparticles without surfactant

10.00 g of $Na_2S_2O_3$.$5H_2O$ dissolved in 50 mL of distilled water then transferred into 250 mL conical flask. A mixture of Sodium thiosulfate was prepared by combining and stirred mechanically at 120 rpm for 40 min and heated in constant bath at 30, 40, 50 and 60 °C, respectively. Then 40 mL of different acid solutions (HCl, HNO_3, H_2SO_4, and H3PO4) were added to the mixture to produce different sized NPs. After the reaction was stopped, the produced yellow precipitates were collected with %yield approximately to (23%), washed with distilled water and then dried.

2.3 Characterization of sulfur nanoparticles

The shape and size characterization of S NPs were conducted using SEM, TEM and XRD techniques.

2.3.1 X-Ray diffraction characterization

X-ray diffraction XRD technique was used to determine the structure and the size of the sulfur nanoparticles, using XRD machine with Cu source (Cu-Kα1 line, λ=1.5045A^{o}).

2.3.2 Scanning electron microscope characterization

The shape and the morphology of the prepared S NPs were characterized by Scanning electron microscope (SEM), the images have obtained using Inspect F50 SEM type.

2.3.3 Transmission electron microscopy (TEM) characterization

TEM technique was used to determine the structure and size of the S NPs using ZEISS EM10CR TEM type.

2.4 Anticancer activity

The cells were maintained in RPMI-1640 or Dulbecco's Minimal Essential Media (DMEM) supplemented with 10% FBS, penicillin (100 U/mL) and streptomycin (100 µg/mL) in a humidified atmosphere of 50 µg/mL CO_2 at 37 °C. Cells which were given as a gift from (HL-60 cell line, DC cell line, HT-29 cell line, Lax cell line).

Leukemia cancer, colon cancer and kidney cancer cell lines were used to test the toxicity of the synthesized S NPs.

2.4.1 In vitro assay for cytotoxicity activity (MTT assay)

The cytotoxicity of samples on various cell types was determined by the MTT assay [75]. Cells (1×10^5/well) were plated in 1 mL of medium/well in 6-well plates (Costar Corning, Rochester, NY). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations 10, 20, 50 and 100 µg/mL of the samples in 0.1% DMSO for 48 h at 37 °C.

After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200 µL/well (5 mg/mL) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide cells (MTT) phosphate-buffered saline solution was added. After 4 h incubation, 0.04 M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570 nm.

Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 570 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks.

The effect of the samples on the proliferation of cell clones was expressed as the % cell viability, using the following formula:

$$\text{Cell viability (\%)} = \frac{A_{570}\,(\text{sample})}{A_{570}\,(\text{control})} \times 100\%$$

To determine the time dependent toxicity of the sample on kidney cell type, concentration of 10 µg/mL was tested for 0, 1, 4,8, 24 and 48 hours post addition of sample to cells at concentration of 1×10^5 µg/mL well. Viability of cells in various wells was determined as described above.

Chapter 3

Results and Discussion

3.1 Sulfur Nanoparticles characterization

The size and the shape of the S NPs were characterized and obtained by using SEM, XRD and TEM.

3.1.1 X-ray characterization of Sulfur Nanoparticles

The X-Ray Diffraction patterns were measured for all prepared S NPs with and without TOAB surfactant are shown in Figure 3.1 to Figure 3.14.

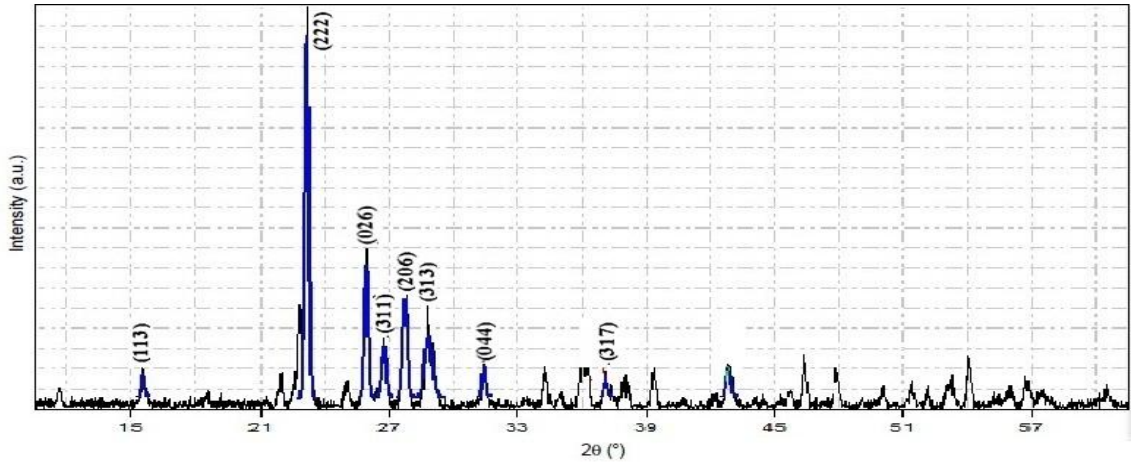

Fig 3.1: XRD pattern of S NPs prepared at 40°C using (0.5 M of HCl) without TOAB surfactant.

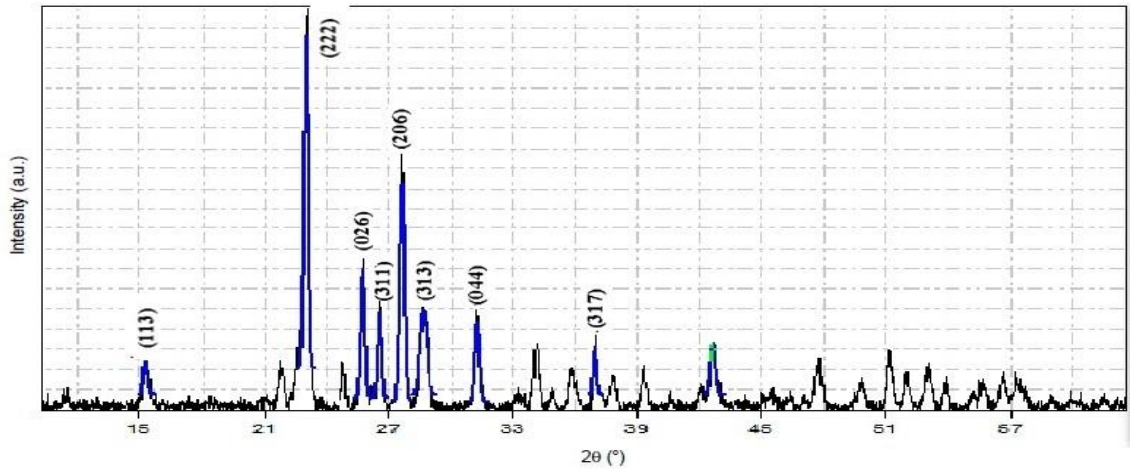

Fig 3.2: XRD pattern of S NPs prepared at 40°C using (1M of HCl) without TOAB surfactant.

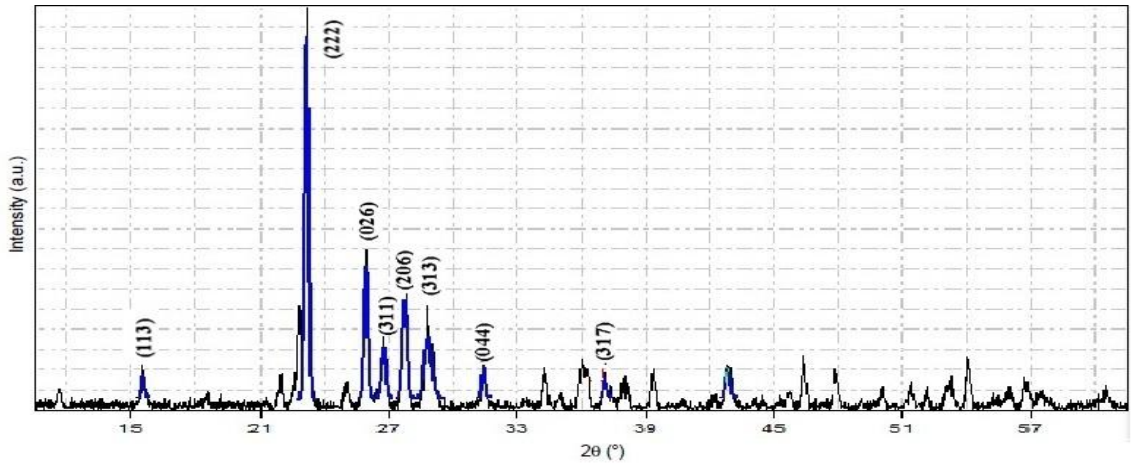

Fig 3.3: XRD pattern of S NPs prepared at 40°C using (2M of HCl) without TOAB surfactant.

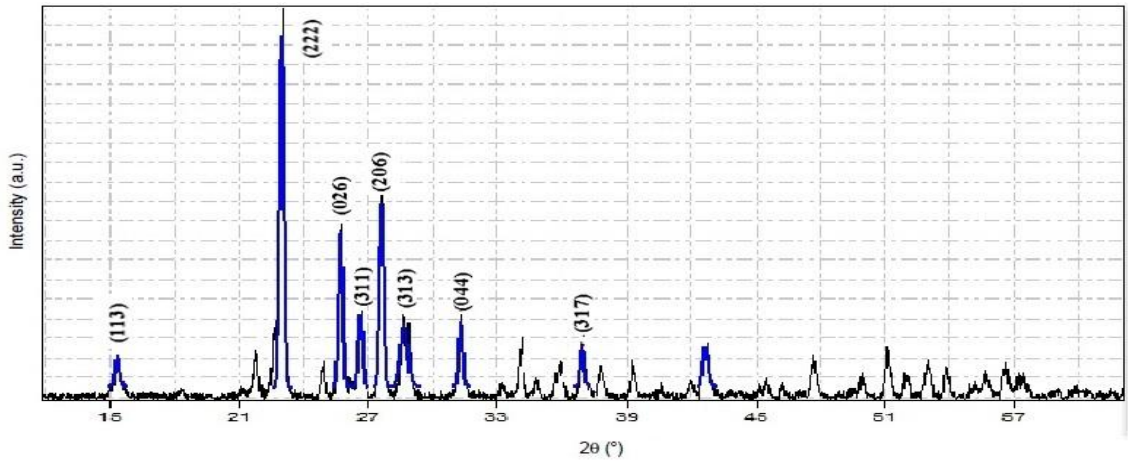

Fig 3.4: XRD pattern of S NPs prepared at 40°C using (3M of HCl) without TOAB surfactant.

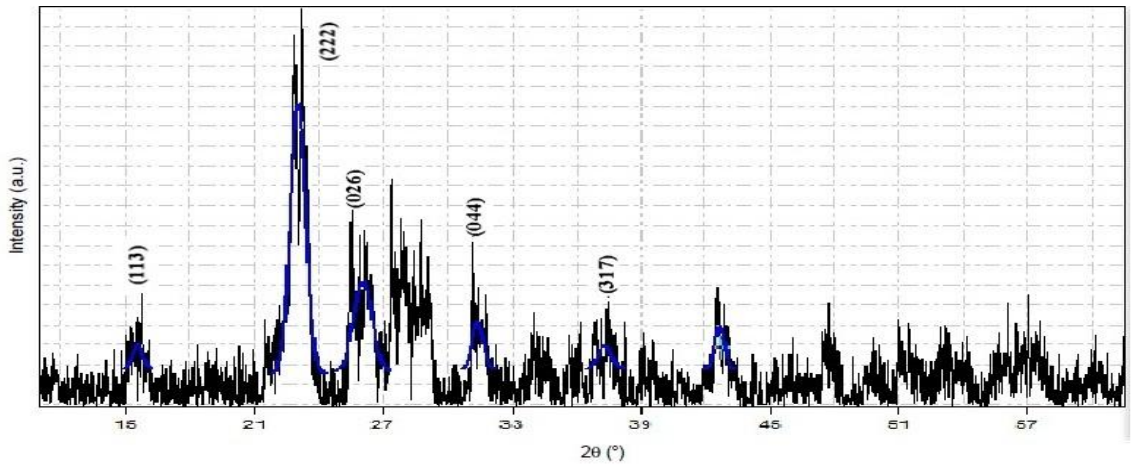

Fig 3.5: XRD pattern of S NPs prepared at 40°C using (0.5 M of HCl) with TOAB surfactant.

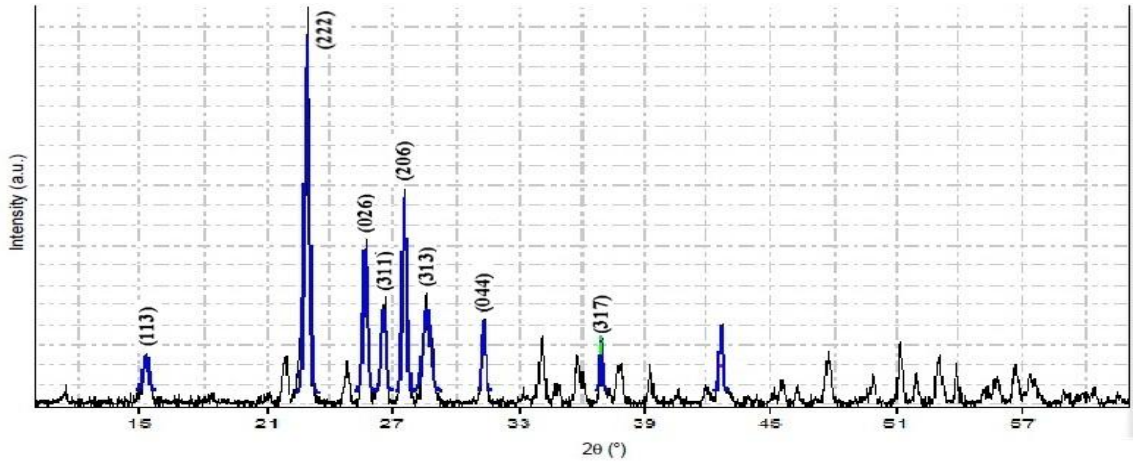

Fig 3.6: XRD pattern of S NPs prepared at 40°C using (1M of HCl) with TOAB surfactant.

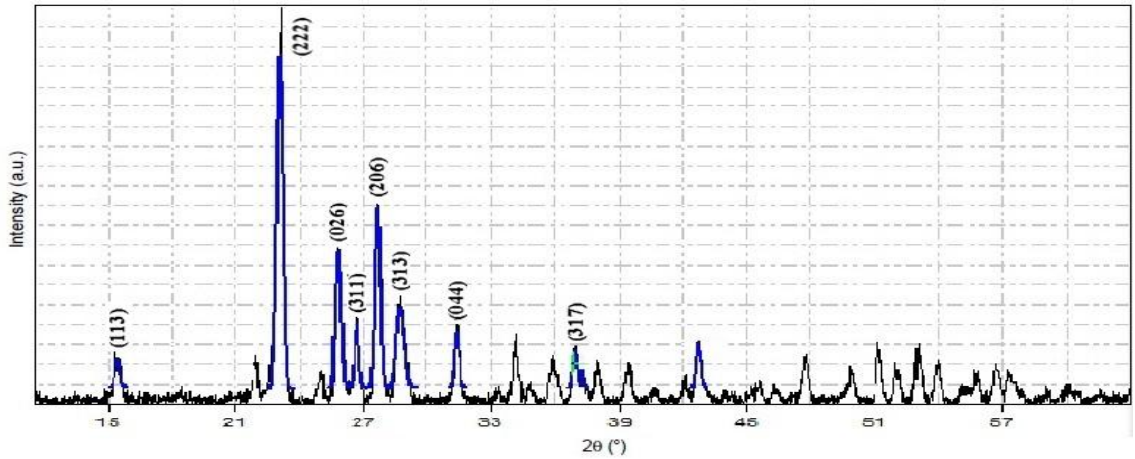

Fig 3.7: XRD pattern of S NPs prepared at 40°C using (2M of HCl) with TOAB surfactant.

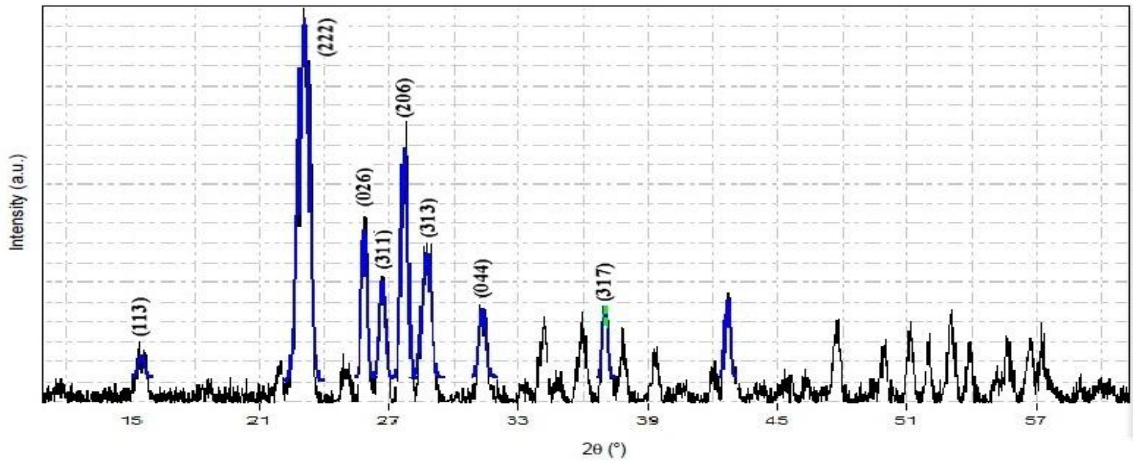

Fig 3.8: XRD pattern of S NPs prepared at 40°C using (3M of HCl) with TOAB surfactant.

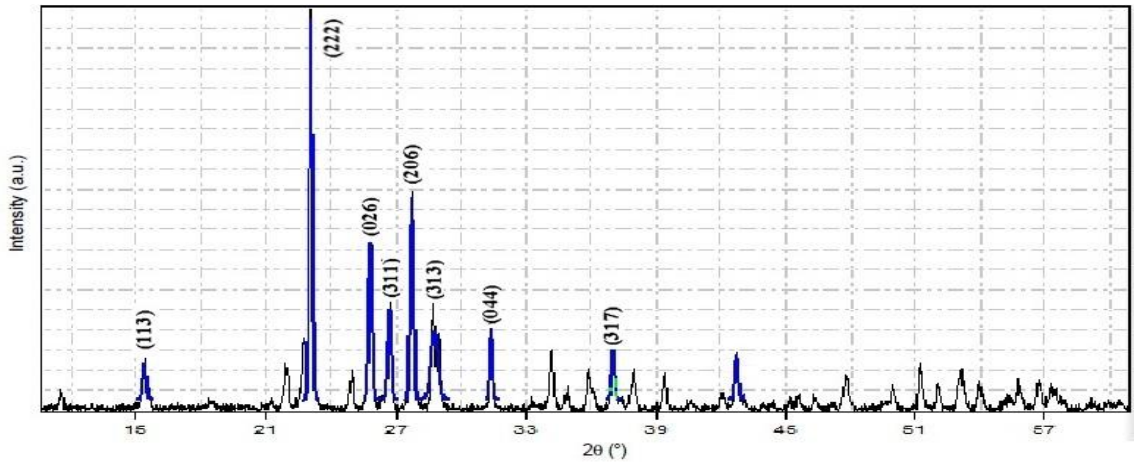

Fig 3.9: XRD pattern of S NPs prepared at 30°C using (2M of HCl) with TOAB surfactant.

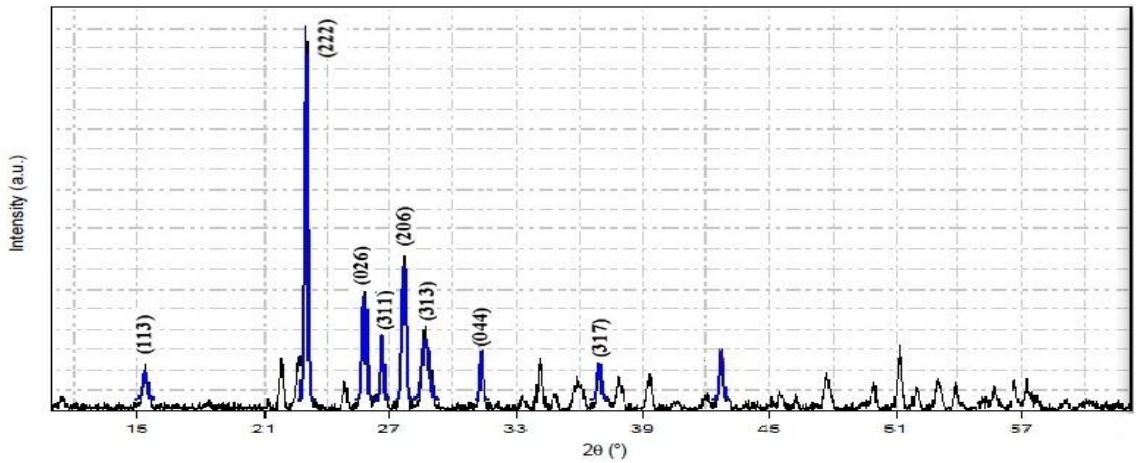

Fig 3.10: XRD pattern of S NPs prepared at 50°C using (2M of HCl) with TOAB surfactant.

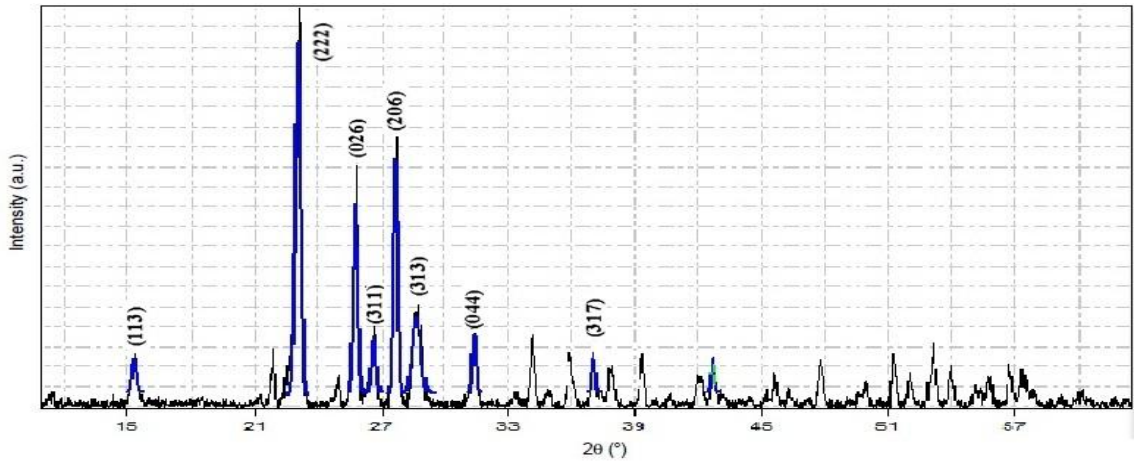

Fig 3.11: XRD pattern of S NPs prepared at 60°C using (2M of HCl) with TOAB surfactant.

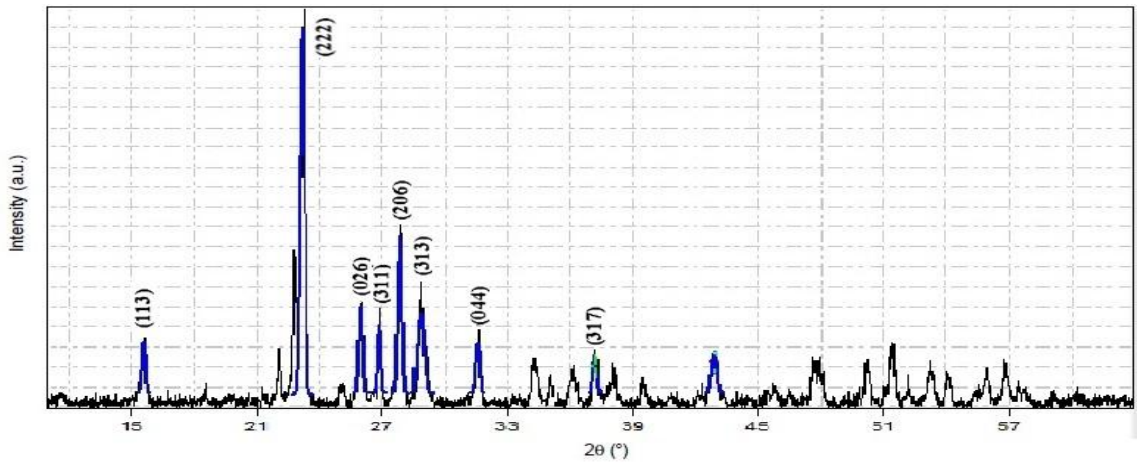

Fig 3.12: XRD pattern of S NPs prepared at 40°C using (2M of HNO_3) with TOAB surfactant.

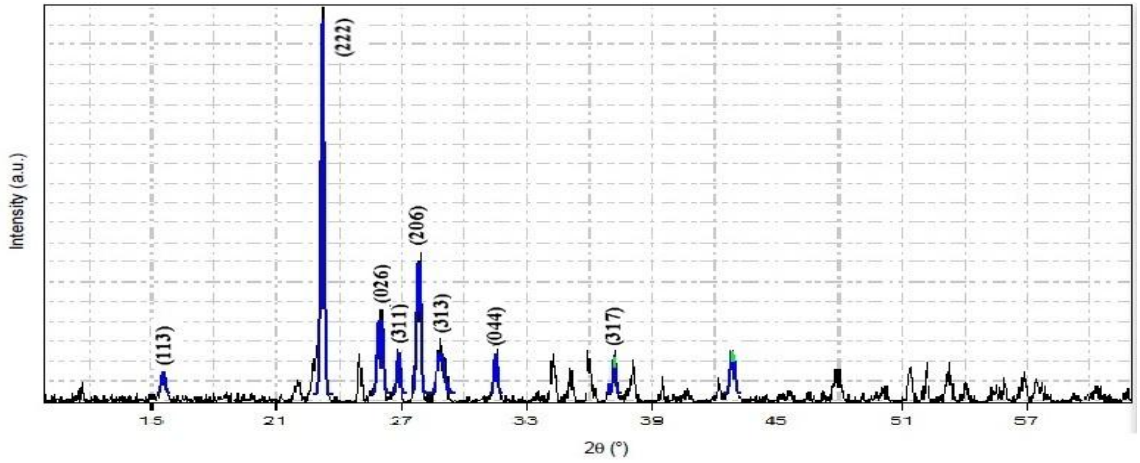

Fig 3.13: XRD pattern of S NPs prepared at 40°C using (2M of H_2SO_4) with TOAB surfactant**.**

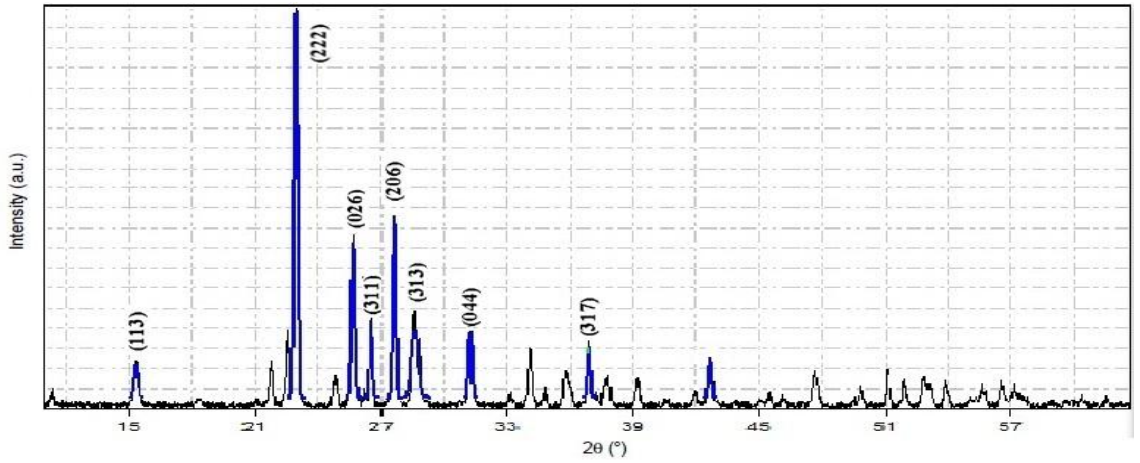

Fig 3.14: XRD pattern of S NPs prepared at 40°C using (2M of H_3PO_4) with TOAB surfactant.

The diffraction peaks were clearly observed from the XRD of the S NPs located near to (15.4, 23.0, 25.8, 26.6, 27.7, 28.8, 31.4 & 37.1) of 2θ positions, that are well-attributed to the (S-(113), S-(222), S-(026), S-(311), S-(206), S-(313), S-(044) & S-(317)) miller planes, respectively .

X-ray structural analysis of the obtained samples show that the samples are S NPs have orthorhombic phase with S_8 structure with traces of monoclinic structure. which was obtained with X. Xie et al. according to JCPDS file No. 83-2285[36].

By applying Scherrer equation on the XRD pattern, the particle size can be calculated:

$$D = K\lambda / (B\cos\theta)$$

Where D is the mean size of crystallites (nm), K is crystallite shape factor a good approximation is 0.9, λ is the X-ray wavelength, *B* is the full width at half the maximum (FWHM) in radians of the X-ray diffraction peak and θ is the Braggs' angle (deg.) [76].

Based on four different XRD peaks analysis, the average size was founded to be 9.2, 10.1, 11.6 and 13.4 nm for TOAB stabilized S NPs which were prepared at 30, 40, 50 and 60 °C, respectively.

These results show that increasing the temperature during nanoparticles preparation lead to increase in nanoparticles size.

The sizes results of sulfur nanoparticles stabilized with TOAB with different temperature are summarized in Table 3.1.

Table 3.1: The sizes using (XRD) of TOAB stabilized S NPs prepared using 2M HCl at different temperatures.

Temperature (°C)	S NPs sizes with surfactant (nm)
30	9.2 (±0.21)
40	10.1 (±0.18)
50	11.6 (±0.15)
60	13.4 (±0.11)

S NPs stabilized with TOAB were prepared at 40 °C using different HCl concentrations (0.5M, 1M, 2M and 3M), the size of the obtained samples was determined from XRD diffraction pattern has found to be 3.4, 8.2, 10.1 and 7.6nm, respectively.

On the other hand, the effect of TOAB surfactant on the NPs sizes was studied. The sizes were found to be 9.8, 10.7, 11.5 and 8.3nm for non-stabilized S NPs which were prepared at 40°C using different HCl concentrations (0.5M, 1M, 2M and 3M), respectively.

This indicates the effect of the stabilizer in preventing the agglomeration of the NPs and hence resulted in smaller sizes at same preparation conditions. The sizes results of S NPs stabilized with TOAB and non-stabilized S NPs prepared at 40°C and by using with different HCl concentration are summarized in Table 3.2.

Table 3.2: The sizes using (XRD) of TOAB stabilized S NPs and nonstabilized S NPs prepared using different HCl concentration at 40°C.

[HCl]	S NPs sizes without surfactant (nm)	S NPs sizes with surfactant(nm)
0.5M	9.8 (±0.12)	3.4 (±0.68)
1M	10.7 (±0.14)	8.2 (±0.16)
2M	11.5 (±0.15)	10.1 (±0.18)
3M	8.3 (±0.14)	7.6 (±0.17)

The effect of the acid type was also studied, for that S NPs stabilized with TOAB were prepared at 40 °C using 2 M solution of different acid (HCl, HNO_3, H_2SO_4 and H_3PO_4). Size analysis using XRD of the obtained samples show that the size is 10.1, 3.9, 5.8 and 6.3 nm for the TOAB stabilized S NPs prepared using 2M solution of (HCl, HNO_3, H_2SO_4 and H_3PO_4), respectively.

The sizes results of TOAB stabilized S NPs with different acid solutions are summarized in Table 3.3.

Table 3.3: The sizes using (XRD) of TOAB stabilized S NPs prepared using 2M solution of different acids at 40°C.

Conc. of acid solution	S NPs sizes with surfactant (nm)
2M of HCl	10.1 (±0.18)
2M of HNO_3	3.9 (±0.52)
2M of H_2SO_4	5.8 (±0.47)
2M of H_3PO_4	6.3 (±0.36)

3.1.2 SEM characterization of Sulfur nanoparticles

The shape and size of NPs were investigated by SEM techniques, Figure 3.15 to Figure 3.28 show the SEM images of all S NPs samples which were prepared with and without surfactant at different temperature, concentration and acid solutions.

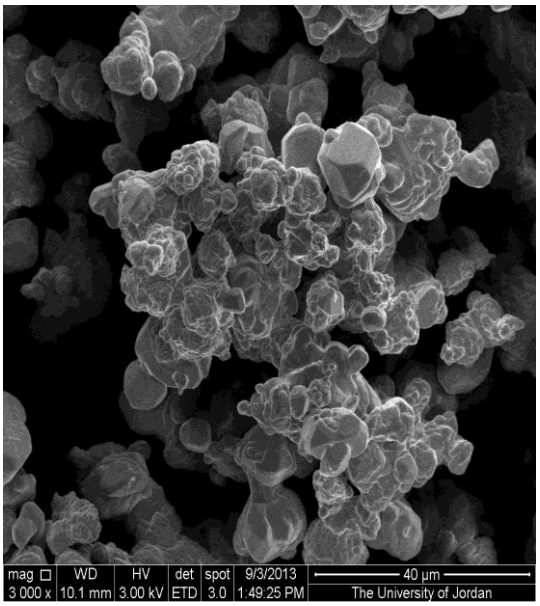

Fig 3.15: The SEM image of nonstabilized S NPs prepared at 40°C using (0.5 M HCl).

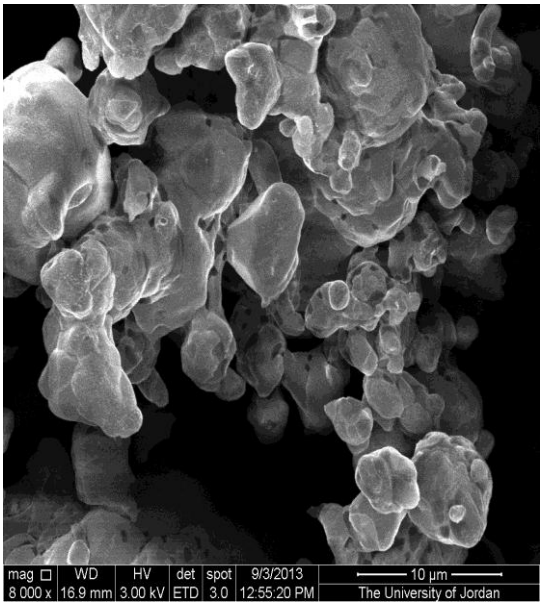

Fig 3.16: The SEM image of nonstabilized S NPs prepared at 40°C using (1 M HCl).

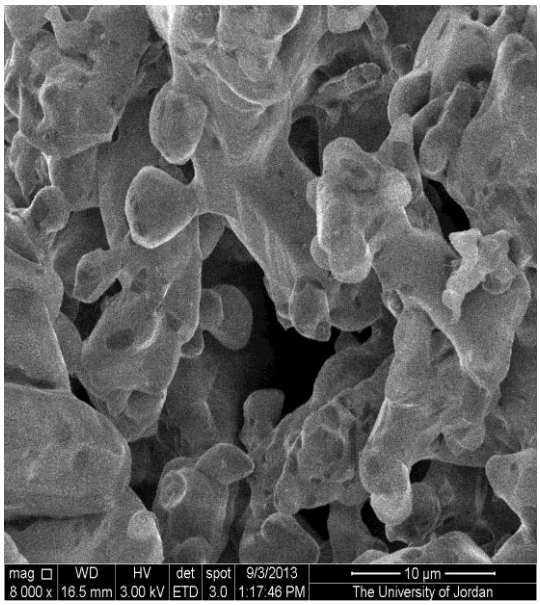

Fig 3.17: The SEM image of nonstabilized S NPs prepared at 40°C using (2 M HCl).

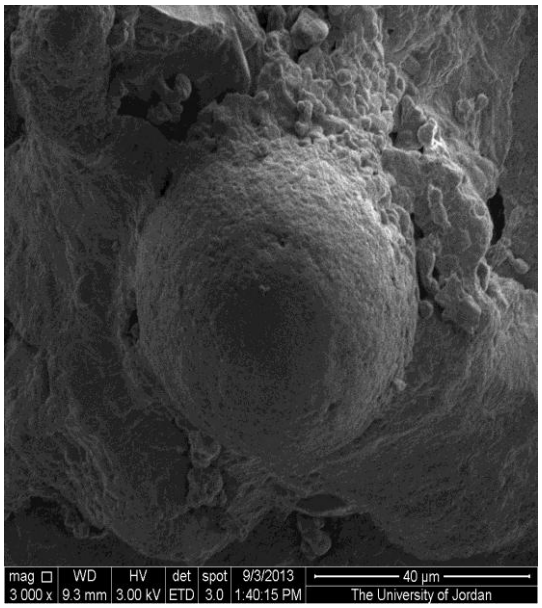

Fig 3.18: The SEM image of nonstabilized S NPs prepared at 40°C using (3 M HCl).

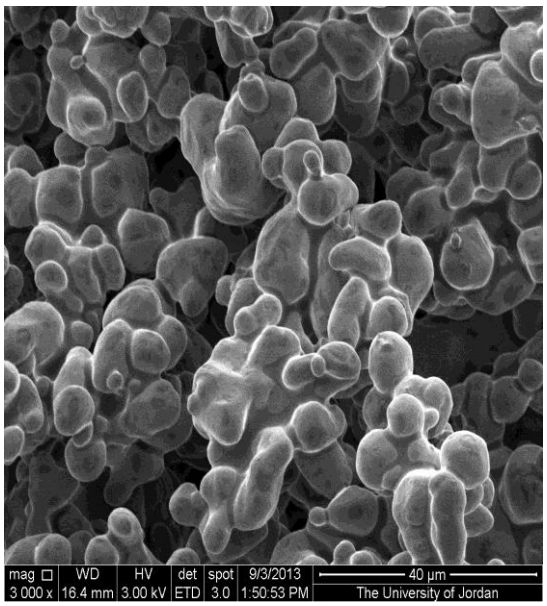

Fig 3.19: The SEM image of TOAB stabilized S NPs prepared at 40°C using (0.5 M HCl).

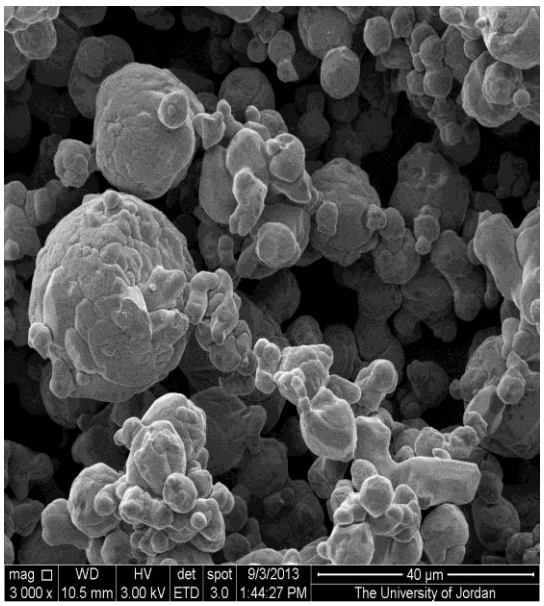

Fig 3.20: The SEM image of TOAB stabilized S NPs prepared at 40°C using (1 M HCl).

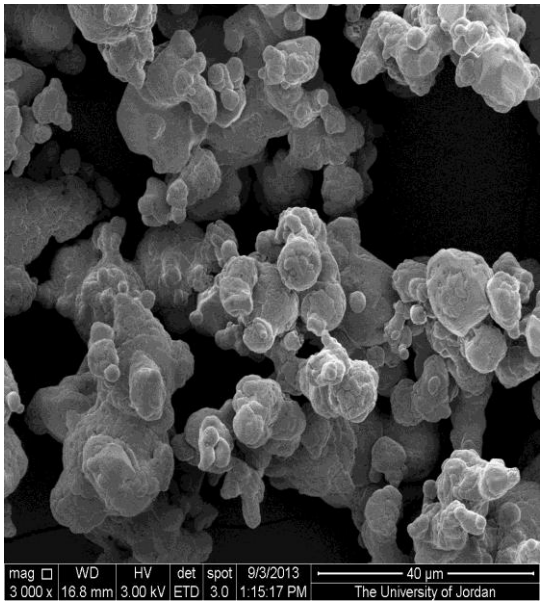

Fig 3.21: The SEM image of TOAB stabilized S NPs prepared at 40°C using (2 M HCl).

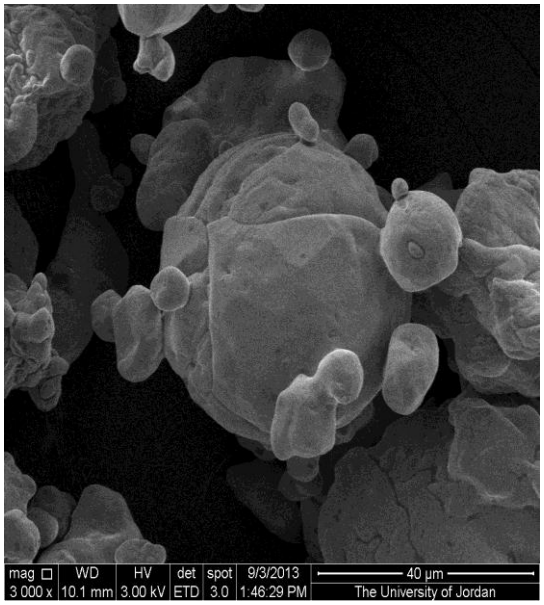

Fig 3.22: The SEM image of TOAB stabilized S NPs prepared at 40°C using (3 M HCl).

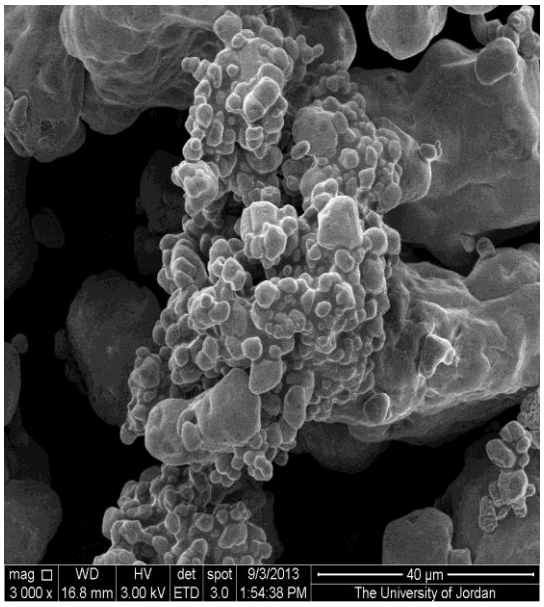

Fig 3.23: The SEM image of TOAB stabilized S NPs prepared at 30°C using (2 M HCl).

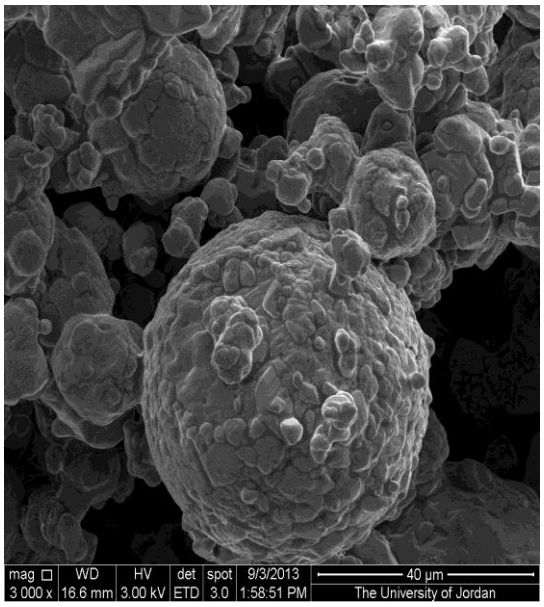

Fig 3.24: The SEM image of TOAB stabilized S NPs prepared at 50°C using (2 M HCl).

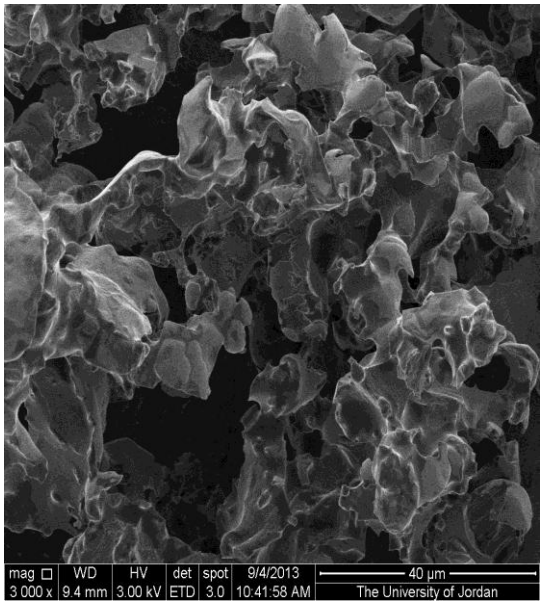

Fig 3.25: The SEM image of TOAB stabilized S NPs prepared at 60°C using (2 M HCl).

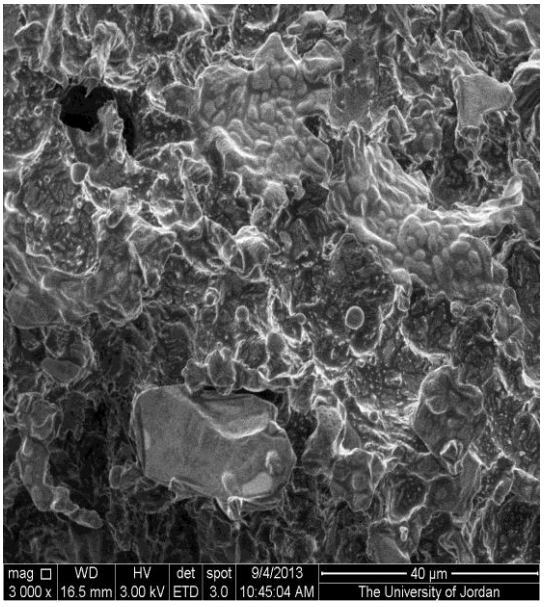

Fig 3.26: The SEM image of TOAB stabilized S NPs prepared at 40°C using (2 M HNO_3).

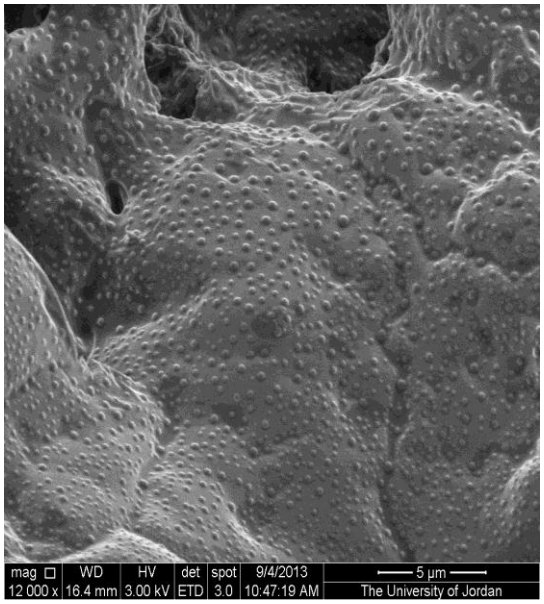

Fig 3.27: The SEM image of TOAB stabilized S NPs prepared at 40°C using (2 M H_2SO_4).

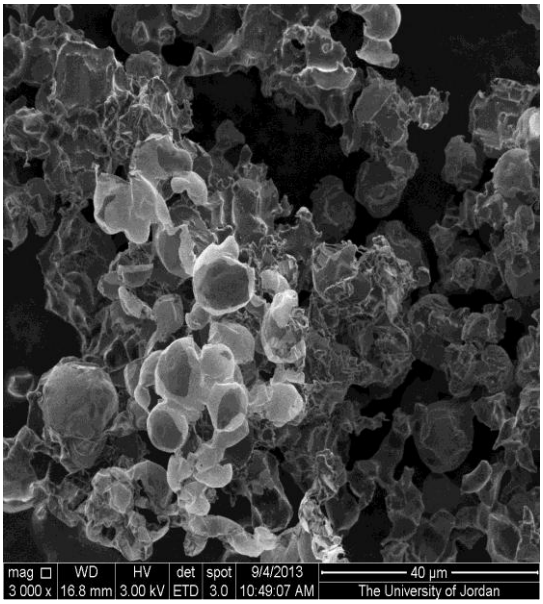

Fig 3.28: The SEM image of TOAB stabilized S NPs prepared at 40°C using (2 M H_3PO_4).

In this work, spherical shape S NPs was obtained for all samples that prepared at different temperatures; 30, 40, 50 and 60 ºC, different concentration of HCl (0.5M, 1M, 2M and 3M) and different acids (HCl, HNO_3, H_2SO_4 and H_3PO_4).

More regular spherical shape was obtained for TOAB stabilized S NPs in compression to the non-stabilized ones. The more regular spherical shape was obtained for TOAB stabilized S NPs at 40 ºC using 0.5M HCl.

3.1.3 TEM characterization of Sulfur nanoparticles

The transmission electron microscopy were measured for all prepared S NPs with and without TOAB surfactant are shown in Figure 3.29 to Figure 3.42.

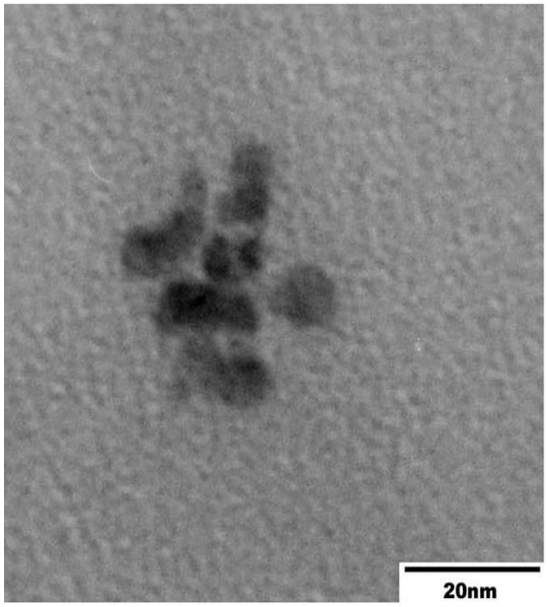

Fig 3.29: The TEM image of nonstabilized S NPs prepared at 40°C using (0.5 M HCl).

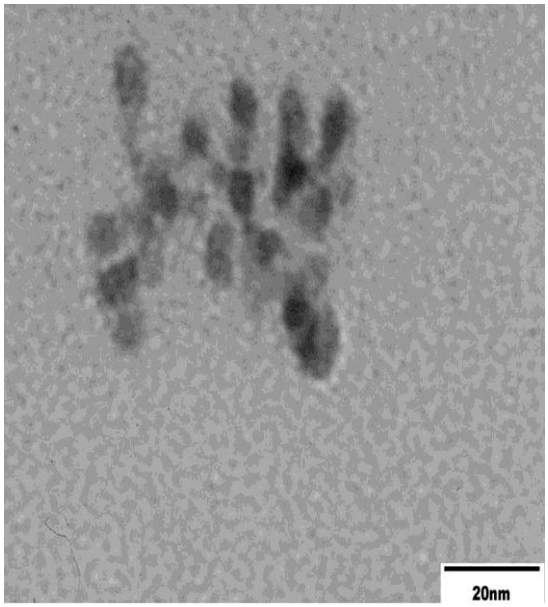

Fig 3.30: The TEM image of nonstabilized S NPs prepared at 40°C using (1 M HCl).

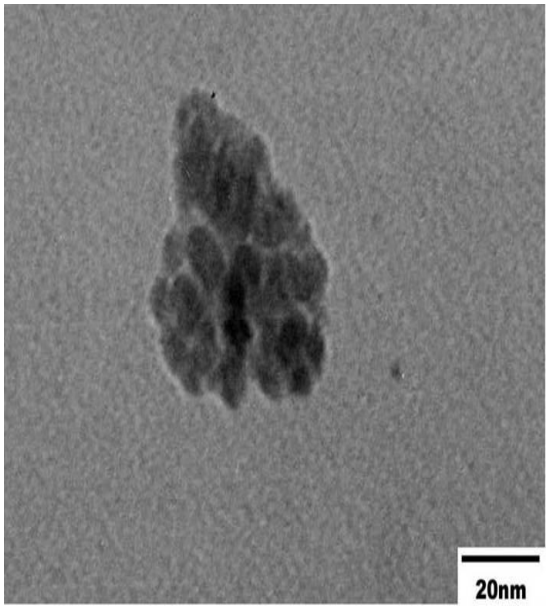

Fig 3.31: The TEM image of nonstabilized S NPs prepared at 40°C using (2 M HCl).

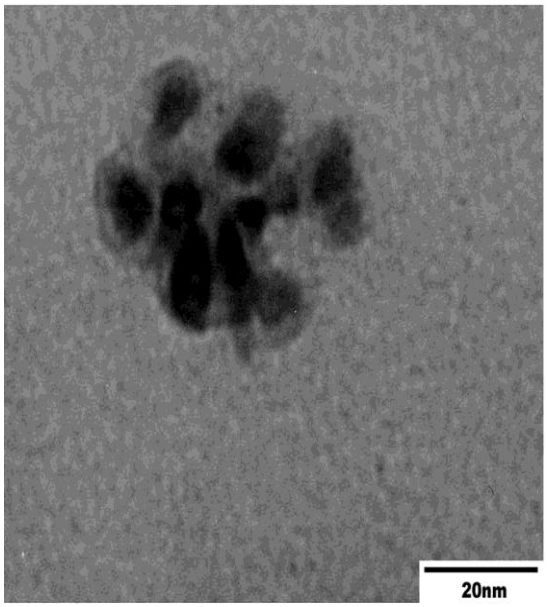

Fig 3.32: The TEM image of nonstabilized S NPs prepared at 40°C using (3 M HCl).

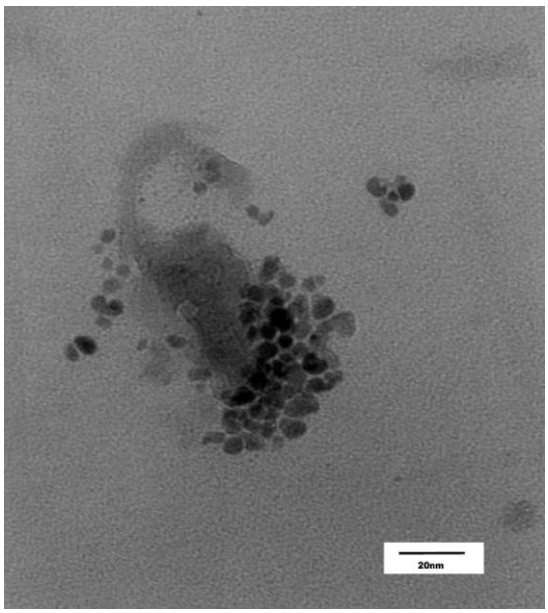

Fig 3.33: The TEM image of TOAB stabilized S NPs prepared at 40°C using (0.5 M HCl).

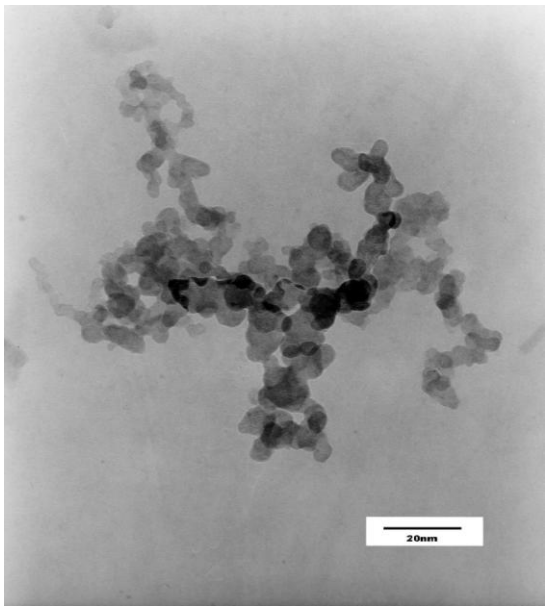

Fig 3.34: The TEM image of TOAB stabilized S NPs prepared at 40°C using (1 M HCl).

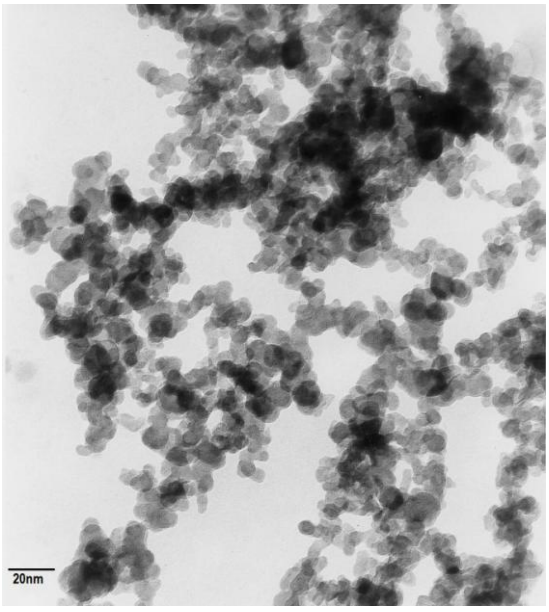

Fig 3.35: The TEM image of TOAB stabilized S NPs prepared at 40°C using (2 M HCl).

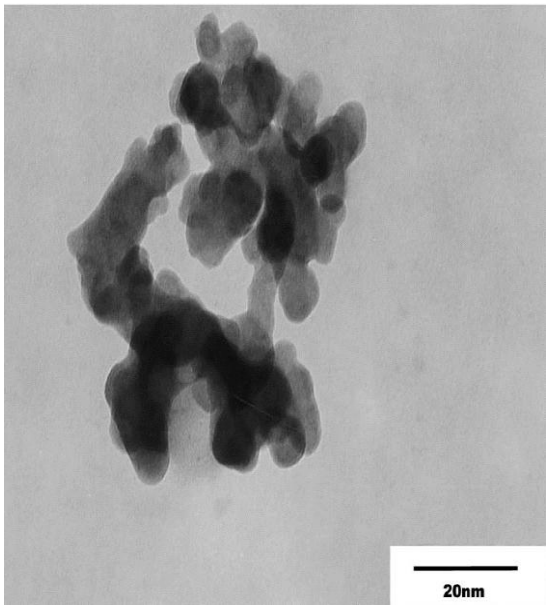

Fig 3.36: The TEM image of TOAB stabilized S NPs prepared at 40°C using (3 M HCl).

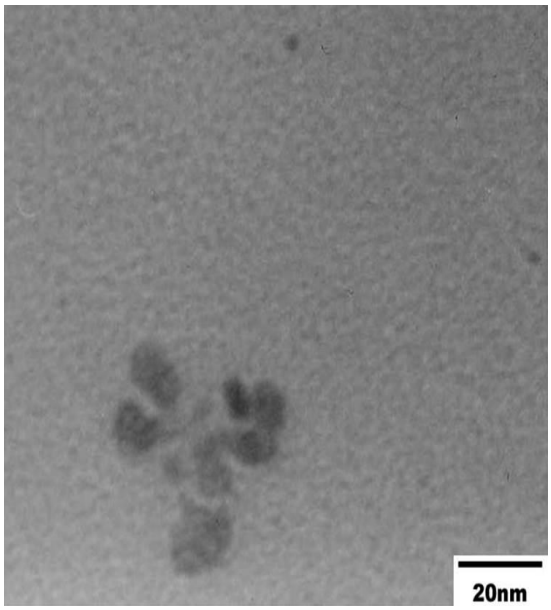

Fig 3.37: The TEM image of TOAB stabilized S NPs prepared at 30°C using (2 M HCl).

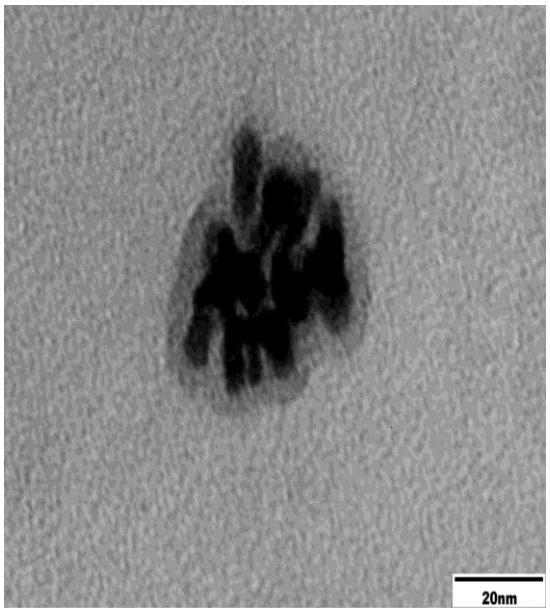

Fig 3.38: The TEM image of TOAB stabilized S NPs prepared at 50°C using (2 M HCl).

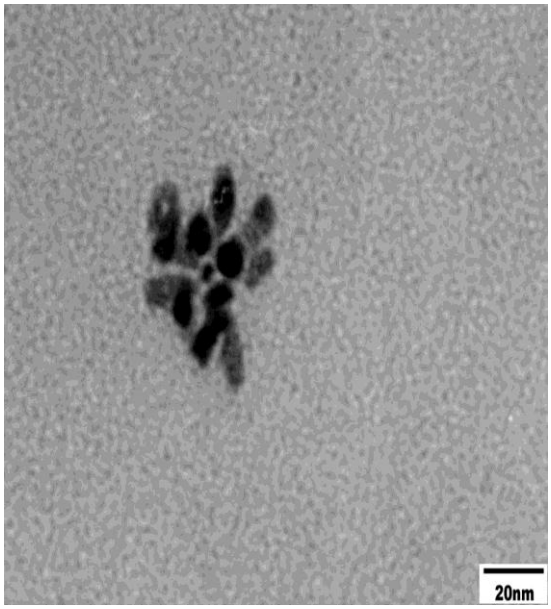

Fig 3.39: The TEM image of TOAB stabilized S NPs prepared at 60°C using (2 M HCl).

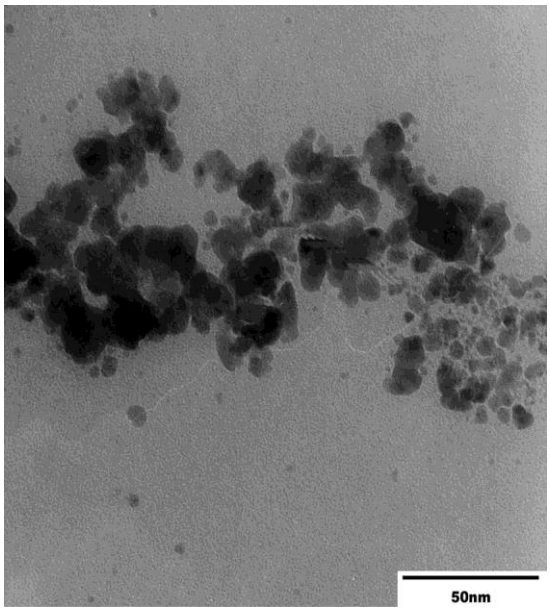

Fig 3.40: The TEM image of TOAB stabilized S NPs prepared at 40°C using (2 M HNO_3).

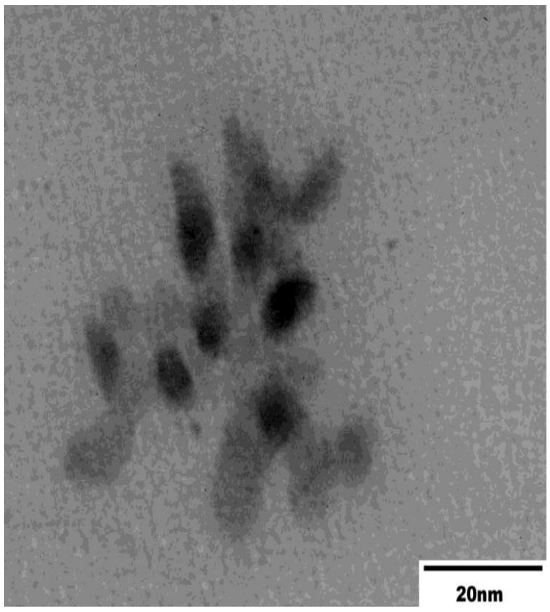

Fig 3.41: The TEM image of TOAB stabilized S NPs prepared at 40°C using (2 M H_2SO_4).

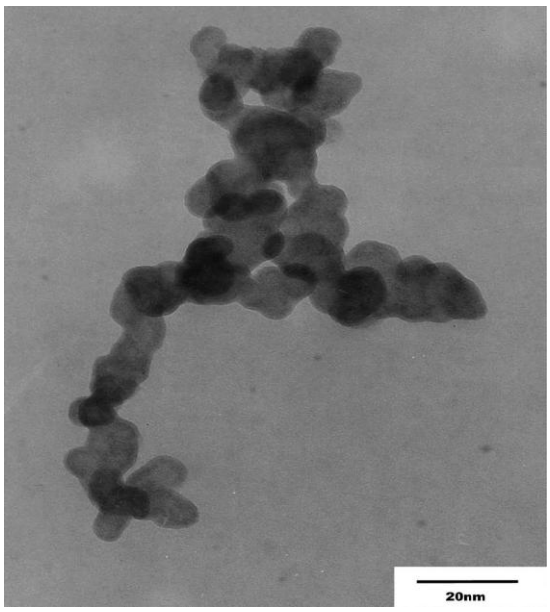

Fig 3.42: The TEM image of TOAB stabilized S NPs prepared at 40°C using (2 M H_3PO_4).

TEM analysis shows that the average sizes are: 6.9, 7.3, 7.6 and 8.2 nm for TOAB stabilized S NPs which were prepared at 30, 40, 50 and 60 °C, respectively using 2M HCl solution.

These results show that increasing the preparation temperature lead to increase in the obtained NPs size. That's mainly explained by two reasons: (i) the acid HCl used in this reaction have endothermic nature, hence their reactivity are increasing which was causing the rising of the diffusion coefficient of sulfur atoms with increasing T [77]. (ii) with increasing temperature the diffusion rate of the new formed atoms increases. Due to these reasons the overall diffusion of sulfur atoms from bulk phase to solid–liquid interface increases, therefore increasing the formed particles sizes [77]. This is in accordance with are results shown in table 3.6.

The sizes of TOAB stabilized S NPs prepared at different temperatures are summarized in Table 3.4.

Table 3.4: The sizes using (TEM) of TOAB stabilized S NPs prepared using 2M HCl at different temperatures.

Temperature (ºC)	S NPs sizes with surfactant (nm)
30	6.9
40	7.3
50	7.6
60	8.2

TOAB stabilized S NPs were prepared at 40 °C using different HCl concentrations (0.5M, 1M, 2M and 3M), the size was determined from TEM was found 3.1, 5.7, 7.3 and 6.1 nm, respectively.

In the other hand, the effect of TOAB surfactant on the NPs sizes was studied. The sizes were found to be 6.1, 6.3, 8.4 and 7.1nm for non-stabilized S NPs prepared at 40°C using different HCl concentrations (0.5M, 1M, 2M and 3M), respectively. The nonstabilized S NPS are obviously larger than TOAB stabilized S NPs prepared at same condition.

The increase in the size with increasing the concentration of the acid can be obtained to an increasing in reaction rate. Therefore, the overall diffusion of sulfur atoms from bulk phase to solid–liquid interface increases, which leads to an increase the particles size [31, 77].

The sizes of TOAB stabilized S NPs and non-stabilized S NPs prepared at 40°C using different HCl concentrations are summarized in Table 3.5.

Table 3.5: The sizes using (TEM) of TOAB stabilized S NPs and nonstabilized S NPs prepared using different HCl concentration at 40°C.

[HCl]	Sizes of nonstabilized S NPs(nm)	Sizes of TOAB stabilized S NPs (nm)
0.5M	6.1	3.1
1M	6.3	5.7
2M	8.4	7.3
3M	7.1	6.1

The sizes of TOAB stabilized S NPs was found 7.3, 2.4, 3.3 and 5.9 nm which were prepared at 40 °C using different acid solutions (HCl, HNO_3, H_2SO_4 and H_3PO_4), respectively.

The increasing orders of particle size in the presence of different acids are: $HNO_3 < H_2SO_4 < H_3PO_4 <$ HCl. The most dependent factor in acid types to the size of the formed S NPs is the ionization constant of the acid, the stronger acid (large ionization constant) has higher reaction rate. Therefore, the denser nuclei are formed and the formed S NPs are with larger size [31].

Based on this role, H_3PO_4 expected to form the less sized particles in comparison to the other used acids, but a contrast behavior is observed, where larger sized nanoparticles are formed. Unlikely, we have not a clear explanation for this behavior. Similar effect was also observed in literature [31], the work of (R. Ghaudhuri et al.) were he has used oxalic acid.

The sizes results of TOAB stabilized S NPs prepared at 40°C using 2M solution of different acids are summarized in Table 3.6.

Table 3.6: The sizes using (TEM) of TOAB stabilized S NPs prepared at 40°C using 2M of different acids.

Conc. of acid solution	S NPs sizes with surfactant (nm)
2M of HCl	7.3
2M of HNO_3	2.4
2M of H_2SO_4	3.3
2M of H_3PO_4	5.9

R. Chaudhuri et al. synthesized S NPs by an organic acid catalyzed precipitation of sodium thiosulphate using different surfactants. The smallest S NPs that was obtained by *Rajab et al*. was 30 nm using CTAB as stabilizer [31].

Y. Guo et al. prepared sulfur NPs via the chemical reaction between sodium polysulfide and hydrochloric acid in a reverse microemulsions system, the average diameter of S NPs was about 20 nm [48].

M. Alexandrovichm et al. Has prepared (22-25 nm average size) S NPs at room temperature by mixing sodium polysulfide aqueous solution with various inorganic and organic acids [78].

M. Shamsipur et al. prepared sulfur NPs by electrochemical method from thiosulfate ion. They show that the particle size of the S-NPs can be adjusted between 35 and 65 nm by varying parameters such as the initial concentration of thiosulfate [50].

With comparing the S NPs that was prepared in this work by quick precipitation method with that prepared by all of *R. Chaudhuri et al.*, *Y. Guo et al.*, *M. Alexandrovichm et al.* and *M. Shamsipur et al.*[31, 48, 78, 50], one can see that in this work S NPs in the range 2.5 to 9 nm can be prepared selectively with relatively narrow size distribution and regular spherical shape .To best of our knowledge these are the smallest S NPs obtained up to now . Moreover, using simple, fast and cheap technique, We large amount of S NPs can be prepared**.**

3.2 Anti cancer activity of sulfur nanoparticles

Anticancer activity of S NPs with TOAB surfactant was studied on leukemia, kidney and colon cancer as well on normal human cell line (Lax Cell). One parameter was studied to obtain the anti cancer activity, this parameter is nanoparticles concentration with size (7.3 nm) of TOAB stabilized S NPs prepared at 40ºC.

3.2.1 Anticancer activity at different concentrations of sulfur nanoparticles with TOAB

The toxicity of different concentrations of samples have been investigated on leukemia, kidney and colon cancer as well on normal human cell lines (Lax Cell).

Table 3.7 shows the effect of S NPs on different cancer cell types. Lax human cell line was used as control.

Table 3.7: Effect of the different concentrations of (7.3 nm) S NPs on various cancer cell types.

Concentrations	0 µg/mL	10 µg/mL	20 µg/mL	50 µg/mL	100 µg/mL
Leukemia cancer	0	0	0	0	15%
Kidney cancer	0	80 %	100%	100%	100%
Colon cancer	0	0	0	20%	40%
Lax Cell	0	0	0	0	0

***(%** that's means percentage of dead cells).

3.2.1.1 Leukemia cancer activity

The obtained results for leukemia cancer activity shows that cancer cells at different concentrations of S NPs do not have any effect on cells at concentrations of 0, 10, 20 and 50 µg/mL after 24 hours. However, at concentration of 100 µg/mL, there was a slight impact of the S NPs, where the cell viability of cancer cells are 85%.

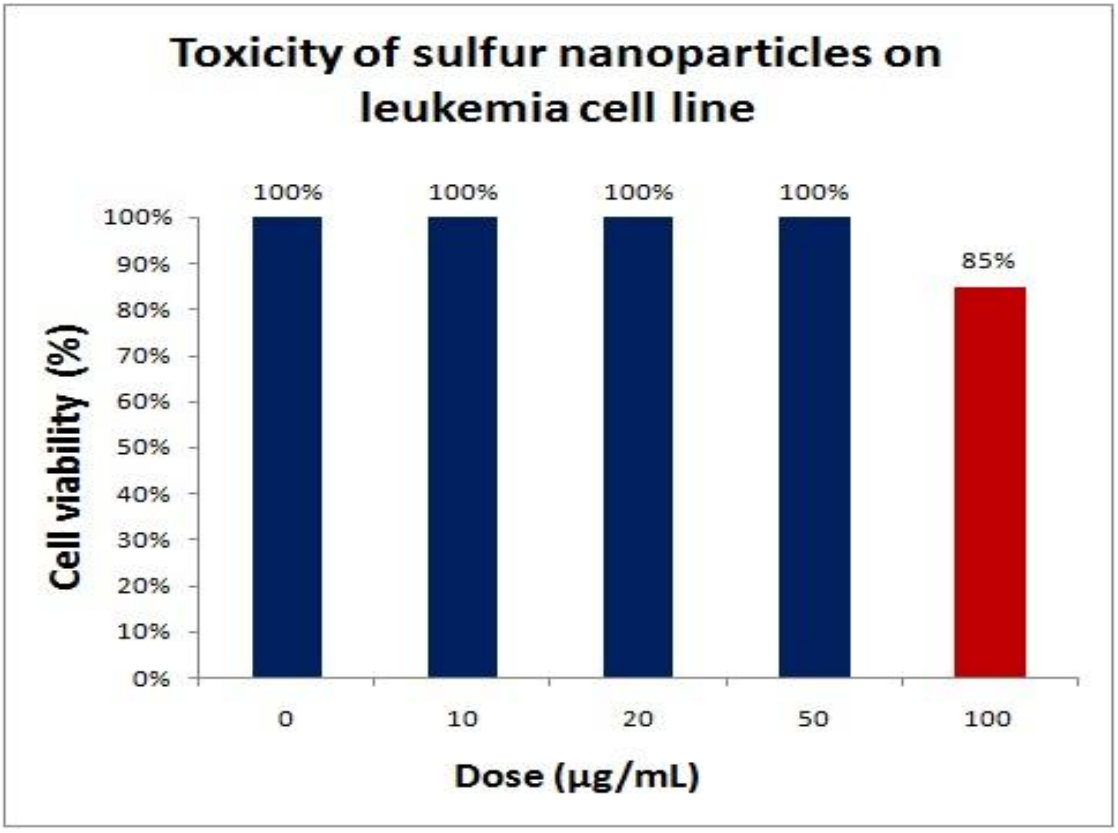

Fig 3.43: Dose-dependent toxicity of 7.3 nm S NPs in Leukemia cells

3.2.1.2 kidney cancer activity

Kidney cells were exposed to (7.3 nm) S NPs at 0, 10, 20, 50, and 100 µg /mL for 24 hours. Cell viability decreased as a function of dosage levels. S NPs showed complete toxicity at concentrations above 10 µg /mL.

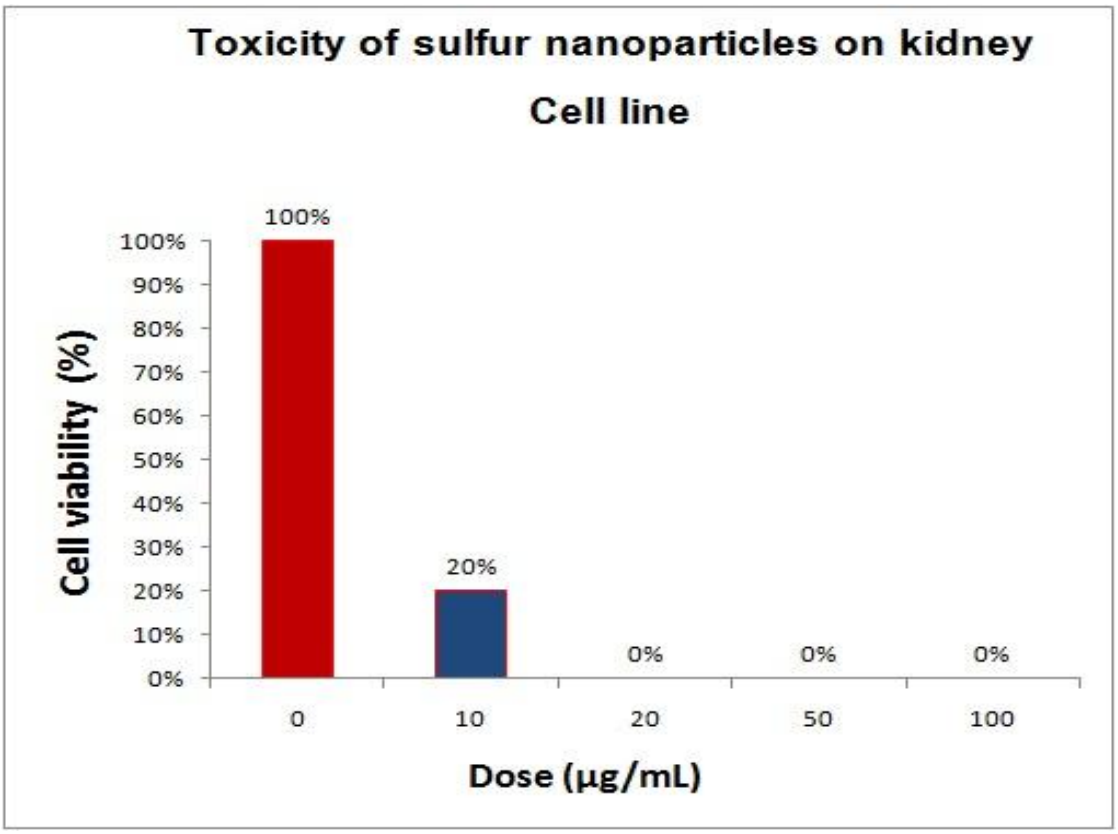

Fig 3.44: Dose-dependent toxicity of (7.3 nm) S NPs in Kidney cells for 24 hr.

3.2.1.3 Time-dependent toxicity of sulfur nanoparticles on kidney cancer cells

To determine the time-dependent toxicity of S NPs on kidney cell lines, 10 µg/mL concentration was incubated with cells as previously shown in materials and methods for 0, 1, 4, 8, 24 and 48 hours.

Figure 3.45 shows that toxicity of S NPs on kidney cell lines is time-dependent. About 50% toxicity is produced by 8 hours.

Figure 3.46 shows kidney cells that are viable after 24 hours. However, figure 3.47 shows affected cells after 24 hours. Normal cell lines are not affected after 24 hours when treated with 100 µg/mL S NPs concentration (Figure 3.49).

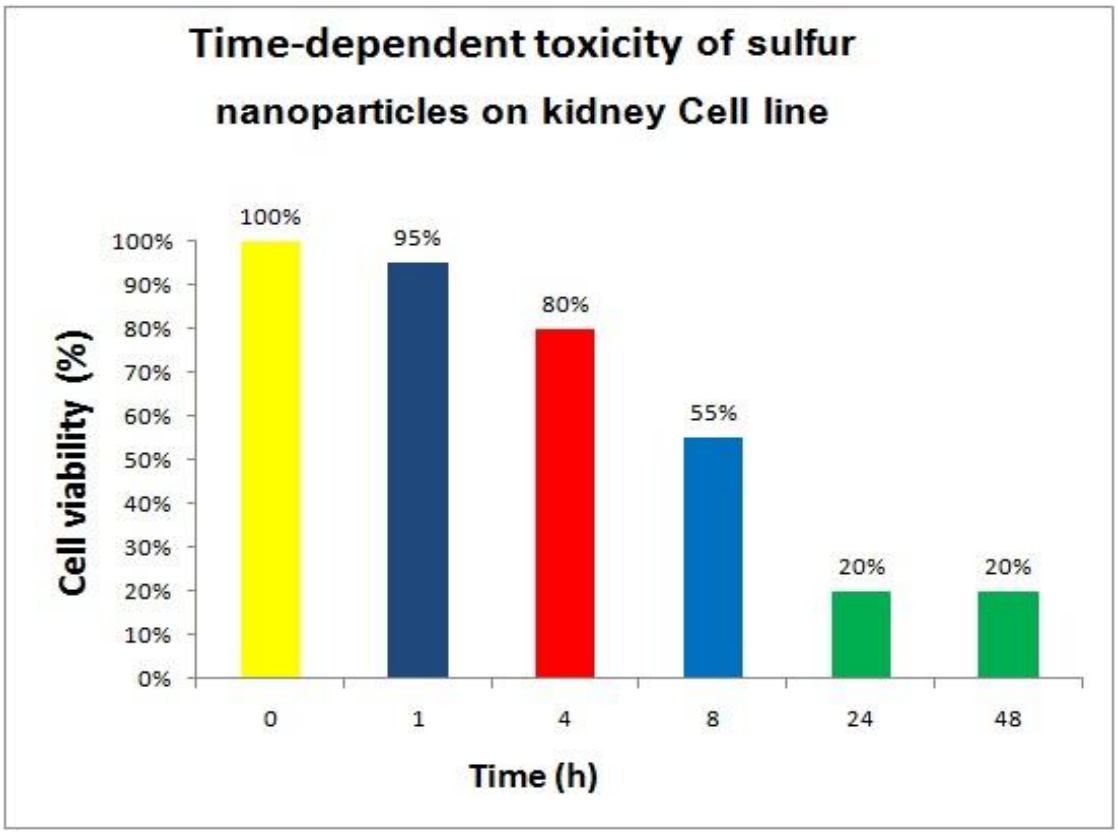

Fig 3.45: Time-dependent toxicity of (7.3 nm) S NPs in Kidney cells.

Our results showed that concentration of 10 µg/mL of S NPs have 80% cytotoxicity on kidney cells. In this regard, F. wanga et al. [71] showed that SiO_2 NPs of (50 nm sized) has 80% toxicity at concentration of 1000 µg/mL (HEK293) cells .

This comparison showed that our S NPs which has 7.3 nm size is 100 times more effective than (F. wanga et al.) preparations [71].

Our investigations show that S NPs prepared in this work are more toxic to kidney cells than leukemia and colon cancer. The reason behind our NPs potency on kidney cell types compared to other cells needs further investigations.

Fig 3.46: The imags of kidney cancer cells before the killing.

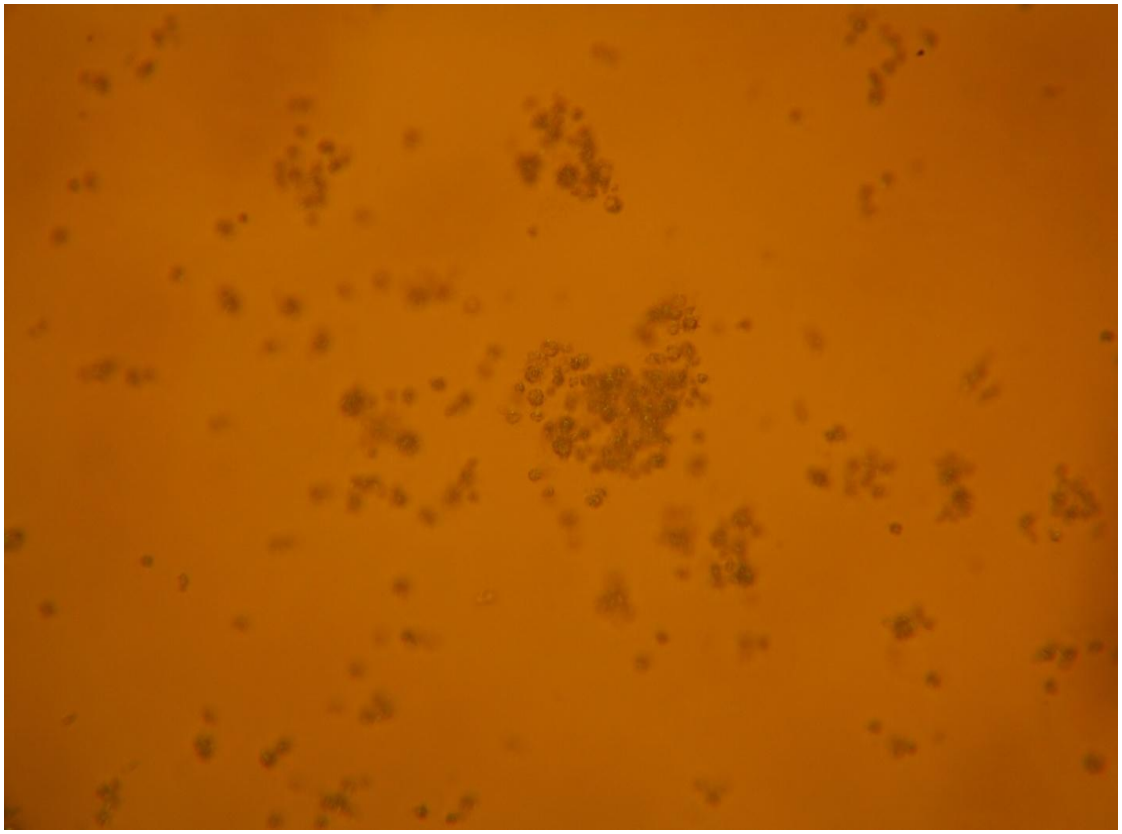

Fig 3.47: The image of kidney cancer cells after the killing at concentration of 10µg/mL

3.2.1.4 Colon cancer activity

The results that obtained for colon cancer activity shows that cancer cells treated with different concentrations of S NPs do not have normal viability at concentrations 0, 10 and 20 µg/mL after 24 hours, but at concentration of 50 and 100 µg/mL there was a slight toxicity of the S NPs, where the cell viabilities of cancer cells were 60% and 80%, respectively.

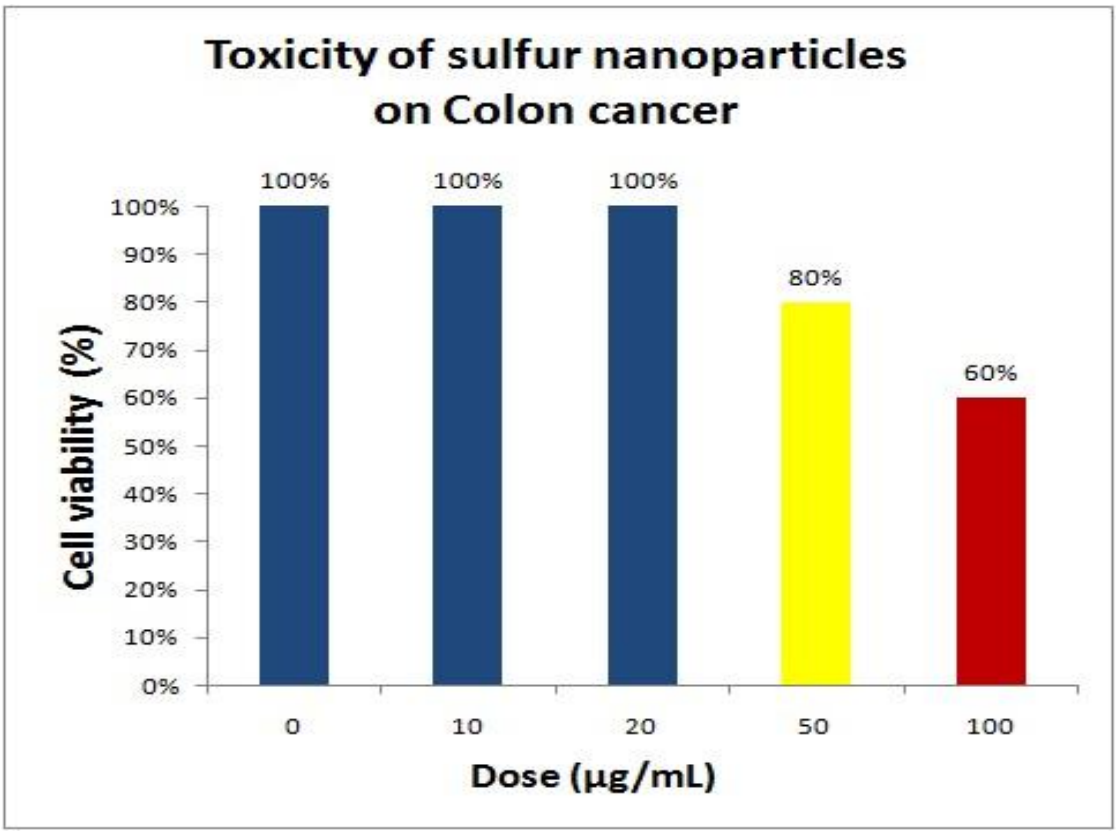

Fig 3.48: Dose-dependent toxicity of (7.3 nm) S NPs in colon cells.

J. Chang et al. [79] showed that silica NPs (21 nm sized) has 20% toxicity at concentration of 667 µg/mL (HT-29) cells.

This comparison showed that our S NPs which has 7.3 nm size is 100 times more effective than (J. Chang et al.) preparations [79]. K. Smitha et al. [80] showed that amorphous chitin NPs (150 nm sized) has 10 % toxicity at concentration of 1 mg/mL (HT-29) cells.

This comparison showed that our S NPs which has 7.3 nm size is 100 times more effective than (K. Smitha et al.) preparations [80].

3.2.3 Effect of sulfur NPs with TOAB on normal cells.

The results that obtained for normal cells (Lax Cell) show that S NPS preparation had no effect on normal cell lines at concentrations 0, 10, 20, 50 and 100 µg/mL after 24 hours. Thus, the later investigation showed that our S NPs are safe on normal cell lines.

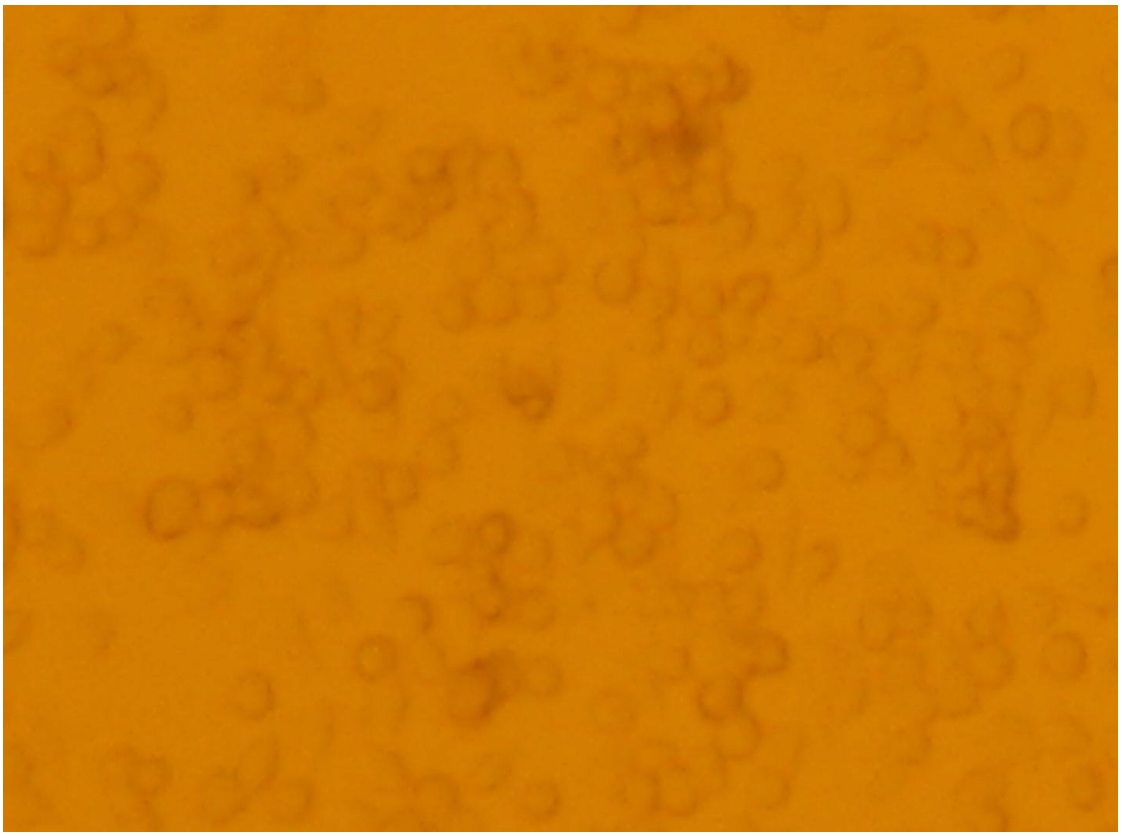

Fig 3.49: The image of S NPs using 10 µg /mL does not have any effect on normal cells.

Conclusions

In this work S NPs with very low dimensional nano-meter-sized less than 10 nm were prepared and investigated against Leukemia, Kidney and Colon cancers, for the first time. The main results obtained in this work can be summarized as:

1- sulfur nanoparticles (S NPs) were successfully prepared by fast, inexpensive and simple quick precipitation method.

2- In this work S NP's were synthesized selectively by varying the preparation parameters: temperature, acid type, acid concentration and the presence of stabilizer.

- The S NPs size increases with increasing the preparation temperature.
- Smaller S NPs sizes were obtained in the presence of TOAB-Stabilizer in comparison to S NP's in absence of stabilizer, at same preparation conditions.
- The maximum and minimum sizes of S NPs were obtained by using HCl and HNO_3, respectively.
- The S NPs size increases with increasing the acid concentration.

3- Spherical shape was recorded for all sulfur NPs with and without tetraoctylammonium bromide (TOAB) surfactant samples. But it looks more regular for sulfur NPs with surfactant, and the same shape for all S NPs prepared at different temperatures; 30, 40, 50 and 60 ºC, different concentrations and different acid solutions.

4- X-Ray diffractograms showed that all obtained sulfur NPs have mainly orthorhombic structure with traces of monoclinic structure.

5- TOAB stabilized S NPs showed higher anticancer activity against kidney cancer.

6- TOAB stabilized S NPs showed a slight impact against leukemia cancer and colon cancer.

7- TOAB stabilized S NPs has no effect on (Lax cell line).

Suggestions for future work

1- Studying the growth kinetic of sulfur nanoparticles at different preparation parameters: temperature, acid type and acid concentration.
2- Studying the anticancer activity of sulfur NPs with other surfactants.
3- Studying the size dependent anticancer activity of S NP's on various cancer cells types.
4- Preparing S NP's using different types of stabilizer and studying there anti cancer activity.
5- In vivo experiments on animals to examine the effectiveness of sulfur NPs.

References

[1] L.Wt. ***Nanoparticles and their biological and environmental applications***. J. of Biosci. Bioeng. 102 (2006) 1–7.

[2] P. Kim, S. Djazayeri, R. Zeineldin. ***Novel nanotechnology approaches to diagnosis and therapy of ovarian cancer.*** Gynecologic Oncology 120 (2011) 393–403.

[3] M. Suleiman, I. Warad, M. Mousa, A. Hussein, B.Hammouti, T.Hadda. ***Copper(II)-Oxide Nanostructures: Synthesis, Characterizations and their Applications–Review.*** J. Mater. Environ. Sci. 4 (2013) 822-827.

[4] P. Mani, R. Srivastava, P. Strasser. ***Dealloyed Pt−Cu Core−Shell Nanoparticle Electrocatalysts for Use in PEM Fuel Cell Cathodes.*** J. Phys. Chem. C, 112 (2008) 2770–2778.

[5] M. Suleiman, N. Jisrawi, O. Dankert, M. Reetz, C. Baehtz, R. Kirchheim, A. Pundt . ***Phase transition and lattice expansion during hydrogen loading of nanometer sized palladium clusters*****.** J. of Alloys and Comp., 356 (2003) 644-648.

[6] H. Hilal, A. Zyoud, N. Zaatar, I. Saadeddin, C. Ali, D. Park, G. Campet. ***CdS-sensitized TiO_2 in phenazopyridine photo-degradation: Catalyst efficiency, stability and feasibility assessment.*** J. of Haza. Mater. 173 (2010) 318-325.

[7] M. Siddiqui, I. warad, S. Adil, R. Mahfouz, A. Al-Arifi. ***Nano-gold Supported Nickel Manganese Oxide: Synthesis, Characterisation and Evaluation as Oxidation Catalyst.*** Oxidation Communications, 35 (2012) 476-481.

[8] V. Georgakilas, D. Gournis, V. Tzitzios, L. Pasquato, D.Guldi, M. Prato. ***Decorating carbon nanotubes with metal or semiconductor nanoparticles.*** J. Mater. Chem., 17 (2007) 2679-2694.

[9] M. Mousa. ***Wastewater Disinfection by Synthesized Copper Oxide Nanoparticles Stabilized with Surfactant***. [Dissertation], An-Najah National University, 2013.

[10] M. Brust , D. Bethell , C.Kiely , D.Schiffrin. ***Self-Assembled Gold Nanoparticle Thin Films with Nonmetallic Optical and Electronic Properties.*** Langmuir, 14 (1998) 5425–5429.

[11] J. Oha, J. Parkb. ***Iron oxide-based super paramagnetic polymeric nanomaterials: Design, preparation, and biomedical application.*** Progress in Polymer Science 36 (2011) 168–189.

[12] R. Mueller, K. Maeder, S. Gohla. ***Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art.*** European J. of Pharma. and Biopharm. 50 (2000) 161-177.

[13] G. Nealon, R. Greget, C. Dominguez, Z. Nagy, D. Guillon, J. Gallani, B. Donnio. ***Liquid-crystalline nanoparticles: Hybrid design and mesophase structures.*** Beilstein J. Org. Chem. 8 (2012) 349–370.

[14] H. Qian, Y. Zhu, R. Jin. ***Atomically precise gold nanocrystal molecules with surface plasmon resonance.*** PNAS , 109 (2012) 696-700.

[15] S. Palchoudhury, Y. Xu, A. Rushdi, R. Holler , Y. Bao. ***Controlled synthesis of iron oxide nanoplates and nanoflowers.*** Chem. Commun.,48 (2012) 499-501.

[16] P. Roy, S. Berger, P. Schmuki. ***TiO_2 Nanotubes: Synthesis and Applications.*** Angewandte Chemie International Edition, 50 (2011) 2904-2939.

[17] D. Tsiourvas, A. Tsetsekou, M. Kammenou, N. Boukos. ***Controlling the Formation of Hydroxyapatite Nanorods with Dendrimers.*** J. of the Amer. Ceramic Soc., 94 (2011) 2023-2029.

[18] M. Abhilash. ***Potential applications of Nanoparticles.*** J. of Pharma and Bio Sci.,1 (2010)1-12.

[19] B. Dabboussi, J.Rodriguez-Viejo, F. Mikulec, J.Hein, H. Mattoussi, R. Ober, K. Jensen, M. Bawendi. ***(CdSe)ZnS Core−Shell Quantum Dots: Synthesis and Characterization of a Size Series of Highly Luminescent Nanocrystallites.*** J. Phys. Chem. 101 (1997) 9463-9475.

[20] A. Faraji, P. Wipf . ***Nanoparticles in cellular drug delivery***. Bioorganic & Medicinal Chemistry 17 (2009) 2950–2962.

[21] M. Guzmán, J. Dille, S. Godet. ***Synthesis of silver nanoparticles by chemicalreduction method and their antibacterial activity.*** Inter. J. of Chem. and Bio. Eng., 2 (2009) 104-111.

[22] C. Welch, R. Compton. ***The use of nanoparticles in electroanalysis: a review.*** Analytical and Bioanalytical Chem., 384 (2006) 601-619.

[23] A. Barakat, M. Al-Noaimi, M. Suleiman, A.Aldwayyan, B. Hammouti, T.Hadda, S.Haddad, A. Boshaala, I. Warad. ***One Step Synthesis of NiO Nanoparticles via Solid-State Thermal Decomposition at Low-Temperature of Novel Aqua(2,9-dimethyl-1,10-phenanthroline)$NiCl_2$ Complex.*** Int. J. of Mol. Sci., 14 (2013) 23941-23954.

[24] R . Mahfouz, K. Al-Khamis, M. Siddiqui, N. Al-Hokbany, I. Warad, N. Al-Andis. ***Kinetic studies of isothermal decomposition of unirradiated and g-irradiated gallium acetylacetonate: new route for synthesis of gallium oxide nanoparticles.*** Progress in Reaction Kinetics and Mechanism, 37 (2012) 249-262.

[25] M. Siddiqui, S. Alshehri, I. Warad, N. El-Salam, R. Mahfouz. ***Model Free Approach for Non-Isothermal Decomposition of Un-Irradiated and γ-Irradiated Silver Acetate: New Route for Synthesis of Ag_2O Nanoparticles.*** Int. J. of Mol. Sci., 11 (2010) 3600-3609.

[26] M. Soleimani, F. Aflatouni, A. Khani. ***A new and simple method for sulfur nanoparticles synthesis.*** Colloid Journal,75 (2013) 112-116.

[27] S. Kulinich, T. Kondo, Y. Shimizu. ***Pressure effect on ZnO nanoparticles prepared via laser ablation in water.*** J. of App. Physics, 113 (2013) 5091-5095.

[28] V. Heredia. ***Silver Nanostructures: Chemical Synthesis of colloids and composites nanoparticles, Plasmon resonance properties and silver nanoparticles monolayer films prepared by spin-coating.*** [DOCTORAL THESIS], Universidad Politécnica de Catalunya , 2011.

[29] W. Jong , P. Borm. ***Drug delivery and nanoparticles: Applications and hazards.*** Int. J. Nanomedicine, 3 (2008) 133–149.

[30] S. Guenter. ***Nanoparticles: from theory to application***. Wiley-VCH, (2011) p.221.

[31] R. Chaudhuri, S. Paria. ***Synthesis of sulfur nanoparticles in aqueous surfactant solutions***. J. Colloid Sci. 343 (2010) 439-446.

[32] S. Al-Thabaiti , F. Al-Nowaiser , A. Obaid , A. Al-Youbi , Z. Khan. ***Formation and characterization of surfactant stabilized silver nanoparticles: A kinetic study."*** Colloids and Surfaces B: Bio interfaces, 67 (2008) 230-237.

[33] T. Reetz, W. Helbig. ***Size-selective synthesis of nanostructured transition metal clusters*.** JACS. , 116 (1994)7401-7402.

[34] A. Pundt , M. Suleiman , C. Bähtz , M. Reetz , R. Kirchheim , N.M. Jisrawi. ***Hydrogen and Pd-clusters.*** Materials Science and Engineering 108 (2004) 19–23.

[35] M. Suleiman, D. Fritsch, C .Borchers, M. Guerdane, A. Pundt. ***Hydrogen absorption in 3.1 nanometre sized palladium samples: does structure matter? .*** International j. of materials research 99 (2008) 528-534.

[36] M. Suleiman, C. Borchers, M. Guerdane, N. Jisrawi, D. Fritsch, R. Kirchheim, A. Pundt. ***Size and Structure of Palladium Clusters Determined by XRD and HREM.*** Zeitschrift fuer Physikalische Chemie, 223 (2009), 169-182.

[37] A. Aldwayyan , F. Al-Jekhedab, M. Al-Noaimi, B. Hammouti, T. Hadda, M. Suleiman, I. Warad. ***Synthesis and Characterization of CdO Nanoparticles Starting from Organometalic Dmphen-CdI2 complex.*** Int. J. Electrochem. Sci., 8 (2013) 10506 – 10514.

[38] W. Cai, T. Gao, H. Hong, J. Sun. ***Applications of gold nanoparticles in cancer nanotechnology.*** Nanotechnology, Science and Applications, 1(2008) 17-32.

[39] J. Ober . ***Materials Flow of Sulfur*** : US Geological Survey Open File Report 02-298, 2003. <http://pubs.usgs.gov/of/2002/of02-298/>.

[40] X. Xie, L. Li, Pu. Zheng, W. Zheng, Y. Bai, T. Cheng, J.Liu. ***Facile synthesis, spectral properties and formation mechanism of sulfur nanorods in PEG-200 .***Materials Res. Bull., 47 (2012) 3665–3669.

[41] Y.An , F.Nie, Z.Wang , D.Zhang. ***Preparation and characterization of realgar nanoparticles and their inhibitory effect on rat glioma cells***. J. of Nano med., 6 (2011) 3187–3194.

[42] I. Porras. ***Sulfur-33 nanoparticles: A Monte Carlo study of their potential as neutron capturers for enhancing boron neutron capture therapy of cancer*** .Applied Radiation and Isotopes, 69 (2011) 1838–1841.

[43] S. Choudhury, S. Roy, A. Goswami, S. Basu. ***Polyethylene glycol-stabilized sulphur nanoparticles: an effective antimicrobial agent against multidrug-resistant bacteria*.** J Anti. Chem. Janua., 67 (2012) 1134-1137.

[44] M. Ellis, D. Ferree, R. Funt, L. Madden. ***Effects of an Apple Scab-Resistant Cultivar on Use Patterns of Inorganic and Organic Fungicides and Economics of Disease Control* .**Plant Dis. 82 (1998) 428.

[45] P. Santiago, E. Carvajal, D.M. Mendoza, L. Rendon. ***Synthesis and Structural Characterization of Sulfur Nanowires.*** Microsc. Microanal. 12(2006) 690.

[46] K. Ghanemi, Y. Nikpour, O. Omidvar. ***Sulfur-nanoparticle-based method for separation and preconcentration of some heavy metals in marine samples prior to flame atomic absorption spectrometry determination.*** Talanta, 85 (2011) 763–769.

[47] M. Suleiman, A. Al Ali, A. Hussein, B. Hammouti, T.Hadda, I.Warad. ***Sulfur Nanoparticles: Synthesis, Characterizations and their Applications.*** J. Mater. Environ. Sci. 4 (2013) 1029-1033.

[48] Y. Guo, J. Zhao, S. Yang, K. Yu, Z.Wang , H. Zhang. ***Preparation and characterization of monoclinic sulfur nanoparticles by water-in-oil microemulsions technique.*** Powder Tech. 162 (2006) 83 – 86.

[49] A. Deshpande, R. Khomane, B. Vaidya, R. Joshi, A. Harle, B. Kulkarni. ***Sulfur Nanoparticles Synthesis and Characterization from H_2S***

Gas, Using Novel Biodegradable Iron Chelates in W/O Microemulsion. Nanoscale Res. Lett. 3 (2008) 221.

[50] M. Shamsipur , S. Pourmortazavi , M. Roushani , I.Kohsari ,S. Hajimirsadeghi***. Novel approach for electrochemical preparation of sulfur nanoparticles.*** Microchim Acta.173(2011) 445–451.

[51] T.Hess , ***Kirk-Othmer Encyclopedia of Chemical Technology*****.** John Wiley & Sons Ltd., New York (2007) p.728

[52] Z. Jia, W. Xu. ***Synthesis and antibacterial activities of quaternary ammonium salt of chitosan*****.** Carbohydrate Research, 333 (2001) 1-6.

[53] A. Jemal, R. Siegel, J. Xu, and E. Ward, "***Cancer statistics, 2010***," CA Cancer Journal for Clinicians, vol. 60, no. 5, pp. 277–300, 2010.

[54] en.wikipedia.org/wiki/Cancer (26 July 2013).

[55] P .Anand, C .Sundaram, K .Harikumar, S .Tharakan, O .Lai, B .Sung. ***Cancer is a preventable disease that requires major lifestyle changes"***. Pharm. Res. 25 (9): 2097–116.

[56] http://www.cancer.gov/cancertopics/cancerlibrary/what-is-cancer (28 July 2013)

[57] S. Nie, Y. Xing, G. Kim, W. Simons. ***Nanotechnology Applications in Cancer.*** Annu. Rev. Biomed. Eng. 92 (2007) 57–88.

[58] J. Walter. ***Leukemia & Lymphoma Society***. New York ,2012.

[59] http://en.wikipedia.org/wiki/Leukemia (27 July 2013)

[60] http://www.webmd.com/cancer/tc/leukemia-topic-overview?page=2 (28 July 2013)

[61]http://www.biologyofhumanaging.com/slides/leukem01. (28July2013)

[62] N. Mylonas. ***Development of positioning devices for MRI-guided high intensity focused ultrasound (HIFU) for abdominal, thyroid and brain, tumours.*** City University London, 2012.

[63] http://www.patient.co.uk/health/kidney-cancer (29 July 2013).

[64] H. Cohen, M. Francis. ***Renal-Cell Carcinoma***. N.Engl J. Med., (2005) 2477-2490.

[65] http://en.wikipedia.org/wiki/Kidney_cancer (29 July 2013).

[66] P. Prakash, M. Porwal, A. Saxena . ***Colon Cancer: General Diagnostic and Treatment.*** J. of Phar. Res. 5 (2012) 355-359.

[67] American Joint Committee on Cancer***. Colon and rectum.*** In: AJCC Cancer Staging Manual. 7th ed. New York: Springer (2010) 143–164.

[68] A.Watson , P.Collins. ***Colon Cancer: A Civilization Disorder.*** Dig. Dis. 29 (2011) 222–228.

[69] J.Mishra, J. Drummond, S.Quazi, S.Karanki, J. Shaw, B.Chen, N. Kumar.***Prospective of colon cancer treatments and scope for combinatorial approach to enhanced cancer cell apoptosis.*** Criti. Rev. in Onco./Hemat. , 86 (2013) 232-250

[70] http://en.wikipedia.org/wiki/File:Colonic_carcinoid_(1)_Endoscopic_resection.jpg (30 July 2013).

[71] F. Wanga , F. Gao , M. Lan, H. Yuan, Y. Huang, J. Liu , ***Oxidative stress contributes to silica nanoparticle-induced cytotoxicity in human embryonic kidney cells*** . Toxicology in Vitro , 23 (2009) 808–815.

[72] S. Gurunathan, J. Han, V. Eppakayala, M. Jeyaraj , J. Kim. ***Cytotoxicity of Biologically Synthesized Silver Nanoparticles in MDA-MB-231 Human Breast Cancer Cells.*** Bio. Res. International , (2013) 1-10.

[73] F.Alexis, E.Pridgen, L.Molnar, O.Farokhzad. ***Factors affecting the clearance and biodistribution of polymeric nanoparticles***. Mol Pharmacol 5 (2008) 05–15.

[74] K.Bae, H.Chung, T.Park. ***Nanomaterials for cancer therapy and imaging***. Mol. Cells 31 (2011) 295–302.

[75] M. Ferrari, M. Fornasiero, A. Isetta. ***MTT colorimetric assay for testing macrophage cytotoxic activity in vitro.*** J. of Immunological Methods, 131 (1990) 165-172.

[76] Holzwarth, Uwe, and Neil Gibson. ***"The Scherrer equation versus the'Debye-Scherrer equation."***Nature Nanotechnology, 69 (2011) 534-534.

[77] R. Chaudhuri, S. Paria. ***Growth kinetics of sulfur nanoparticles in aqueous surfactant solutions.*** J. of Colloid and Inter. Sci. 354 (2011) 563–569.

[78] M. Alexandrovichm , M. Gazizyanovich, S. Rifhatovna, K. Nailevich, Z. Maratovna. ***Obtaining Sulfur Nanoparticles from Sodium Polysulfide Aqueous Solution.*** J. Chem. Chem. Eng., 6 (2012) 233-241.

[79] J. Chang, K. Chang , D. Wang , Z. kong. ***In Vitro Cytotoxicitiy of Silica Nanoparticles at High Concentrations Strongly Depends on the Metabolic Activity Type of the Cell Line.*** Environ. Sci. Technol., 41 (2007) 2064–2068.

[80] K. Smitha, A. Anitha, T. Furuike, H. Tamura, S. Naira, R. Jayakumar. ***In vitro evaluation of paclitaxel loaded amorphous chitin nanoparticles for colon cancer drug delivery.*** Colloids and Surfaces B: Biointerfaces 104 (2013) 245– 253.

كلية الدراسات العليا

جامعة النجاح الوطنية

تحضير حبيبات نانومترية من الكبريت وبحث فعاليتها ضد الخلايا السرطانية

إعداد

انس خالد عبد العلي

إشراف

د. محمد سليمان

د. ايمن حسين

قدمت هذه الأطروحة استكمالاً لمتطلبات درجة الماجستير في الكيمياء بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.

2013

تحضير حبيبات نانومترية من الكبريت وبحث فعاليتها ضد الخلايا السرطانية

إعداد

انس خالد عبد العلي

إشراف

د. محمد سليمان

د. ايمن حسين

الملخص

حبيبات النانو هي عبارة عن تجمع عدد صغير من الذرات لعنصر ما، بحيث يشكل مجموع هذه الذرات جزئيا يقل حجه عن 100 نانومتر. ما يميز هذه الجزئيات ان لها خصائص كيميائية وفيزيائية مختلفة عن الخصائص التي تمتلكها في حال كانت الجزئيات كبيرة، وذلك بسبب الزيادة الكبيرة في سطحها مقارنة مع مساحة سطح الجزئيات الكبيرة .

في هذه الدراسة تم تحضير الكبريت بحجم النانو بالشكل الكروي وبعدة أحجام مختلفة واستخدامها كمضاد للسرطان. الطريقة التي استخدمت في تحضير الكبريت بحجم النانو هي ترسيب الكبريت على درجات حرارة مختلفة 30 ، 40، 50 ، 60 س° واستخدام تراكيز مختلفة من HCl و استخدام أحماض مختلفة مثل (H_2SO_4 ,HNO_3, H_3PO_4) وكذلك تم تغليف الكبريت باستخدام مثبت سطحي .

وقد تم تشخيص الكبريت بحجم النانو لمعرفة حجم الجزئيات وشكلها باختلاف درجة حرارة التحضير واختلاف التراكيز وأحماض مختلفة وتأثير وجود المثبت على الحجم والشكل. لتشخيص الكبريت المحضر بحجم النانو، استخدمت الاجهزة التالية : TEM و SEM و XRD.

أظهرت النتائج ان حجم جزيئات النانو المُحَضَّرة كانت تتراوح بين 2 و 9 نانومتر، بحيث كانت العلاقة طردية مع ارتفاع درجة حرارة التحضير بحيث أثبتت الدراسة انه كلما ازدادت درجة حرارة التحضير كانت جزيئات النانو بحجم اكبر، وكذلك كانت العينات المثبتة باستخدام

المذيب السطحي اصغر حجما من العينات غير المثبتة عند نفس درجات الحرارة .وكذلك فان جميع العينات المُحَضَّرة كانت بالشكل الكروي.

تم اختيار حجم واحد من الكبريت المحضر بحجم النانو (7.3 نانومتر) لدراسته على خلايا سرطان الدم وسرطان الكلى وسرطان القولون وكذلك تم دراسة الكبريت بحجم النانو على الخلايا الحية لمعرفة تأثيره عليها.

أظهرت الدراسة فعالية قوية للكبريت بحجم النانو المغلف بالمواد الفعالة سطحيا ضد سرطان الكلى وكان هناك تأثير طفيف على سرطان الدم وسرطان القولون على تراكيز 50 µg/mL و 100 µg/mL.

الكبريت بحجم النانو كان له تأثير قوي وفعال ضد خلايا سرطان الكلى حيث كانت نسبة القتل للخلايا السرطانية على تراكيز مختلفة 20 µg/mL و 50 µg/mL و100 µg/mL هي100%، لكن على اقل تركيز وهو 10 µg/mL كانت نسبة القتل للخلايا السرطانية هي 80 %. وكذلك تم دراسة تأثير الوقت على خلايا سرطان الكلى على تركيز 10 µg/mL حيث كانت نسبة القتل للخلايا السرطانية بعد 24 ساعة هي 80 % وهذا دليل على مدى فعالية وتأثير الكبريت بحجم النانو ضد خلايا سرطان الكلى.

وللتأكد من أن الكبريت بحجم النانو ليس له تأثير على الخلايا الحية في جسم الإنسان تمت دراسته على نوع من الخلايا المعروفة بـــ (Lax cell) على تراكيز مختلفة 10 µg/mL و 20µg/mL و 50 µg/mL و 1000 µg/mL حيث تبين انه ليس له أي تأثير على الخلايا الحية.