CHAPTER ONE

Introduction

1.Introduction

1. 1 Overview

Nanotechnology is the ability to measure, design, and manipulate at the atomic, molecular and supramolecular levels on a scale of about 1 to 100 nm in an effort to understand, create, and use material structures, devices, and systems with fundamentally new properties and functions attributable to their small structures [1]. All biological and man-made systems have their first levels of organization at the nanoscale (nanocrystals, nanotubes, and nanobiomotors), where their fundamental properties and functions are defined. The goal in nanotechnology may be described as the ability to assemble molecules into useful objects hierarchically integrated along several length scales and then, after use, disassemble objects into molecules. Nature already accomplishes this in living systems and in the environment.

Nanobiomedicine is a field that applies nanoscale principles and techniques to understanding and transforming inert materials and biosystems (nonliving, living or thinking) for medical purposes such as drug synthesis, brain understanding, body part replacement, visualization, and tools for medical interventions. Integration of nanotechnology with biomedicine and biology, and with information technology and cognitive science is expected to accelerate in the next decade [2]. Convergence of nanoscale science with modern biology and medicine is a trend that should be reflected in science policy decisions [3].

Nanobiosystem science and engineering is one of the most challenging and fastest growing components of nanotechnology. It is essential for better understanding of living systems and for developing new tools for medicine and solutions for health care (such as synthesis of new drugs and their targeted delivery, regenerative medicine, and neuromorphic engineering). One important challenge understands the processes inside cells and neural systems. Nanobiosystems are sources of inspiration and provide models for man-made nanosystems. Research may lead to better biocompatible materials and nanobiomaterials for industrial applications. The confluence of biology and nanoscience will contribute to unifying concepts of science, engineering, technology, medicine, and agriculture [4].

1.2 Nanoparticles and their properties

A nanoparticle is by definition a particle where all the three dimensions are in nanometer scale [5]. These particles exhibit electronic, optical, magnetic and chemical properties that are very different from both the bulk and the constituent atoms or molecules [6, 7].

Nanoparticles cover a broad area of interest including electronics, medicine, food industry, environmental applications and cosmetics [8].

1.2.1 Nanoparticle advantages

Nanoparticle, crystal and nanolayer manufacturing processes aim to take advantage of four kinds of effects:

a) New physical, chemical or biological properties are caused by size scaling. Smaller particle size determines larger interfacial area, an increased number of molecules on the particle interfaces, quantum electromagnetic interactions, increased surface tension, and size confinement effects (from electronic and optic to confined crystallization and flow structures). The wavelike properties of the electrons inside matter are affected by shape and volume variations on the nanometer scale. Quantum effects become significant for organizational structures under 50 nm, and they manifest even at room temperature if their size is less than 10 nm.

b) New phenomena are due to size reduction to the point where interaction length scales of physical, chemical and biological phenomena (for instance, the magnetic, laser, photonic, and heat radiation wavelengths) become comparable to the size of the particle, crystal, or respective microstructure grain.

c) Generation of new atomic, molecular and macromolecular structures of materials by using various routes: chemistry (three-dimensional macromolecular structures, chemical self assembling), nanofabrication (creating nanostructures on surfaces, manipulation of three-dimensional structures), or biotechnology (evolutionary approach, bio-templating, and three-dimensional molecular folding).

d) Significant increase of the degree of complexity and speed of processes in particulate systems. Time scales change because of smaller distances and the increased spectrum of forces with intrinsically short time scales (electrostatic, magnetic, electrophoresis, radiation pressure, others) [9].

1.2.2Nanoparticles shapes

Nanoparticles are known to exist in diverse shapes such as spherical, triangular, cubical, pentagonal, rod-shaped, shells, ellipsoidal and so forth. Nanoparticles by themselves and

when used as building blocks to construct complex nanostructures such as nanochains, nanowires, nanoclusters and nanoaggregates find use in a wide variety of applications in the fields of electronics, chemistry, biotechnology and medicine, just to mention few: For example, gold nanoparticles are being used to enhance electrolumin escence and quantum efficiency in organic light emitting diodes [5].

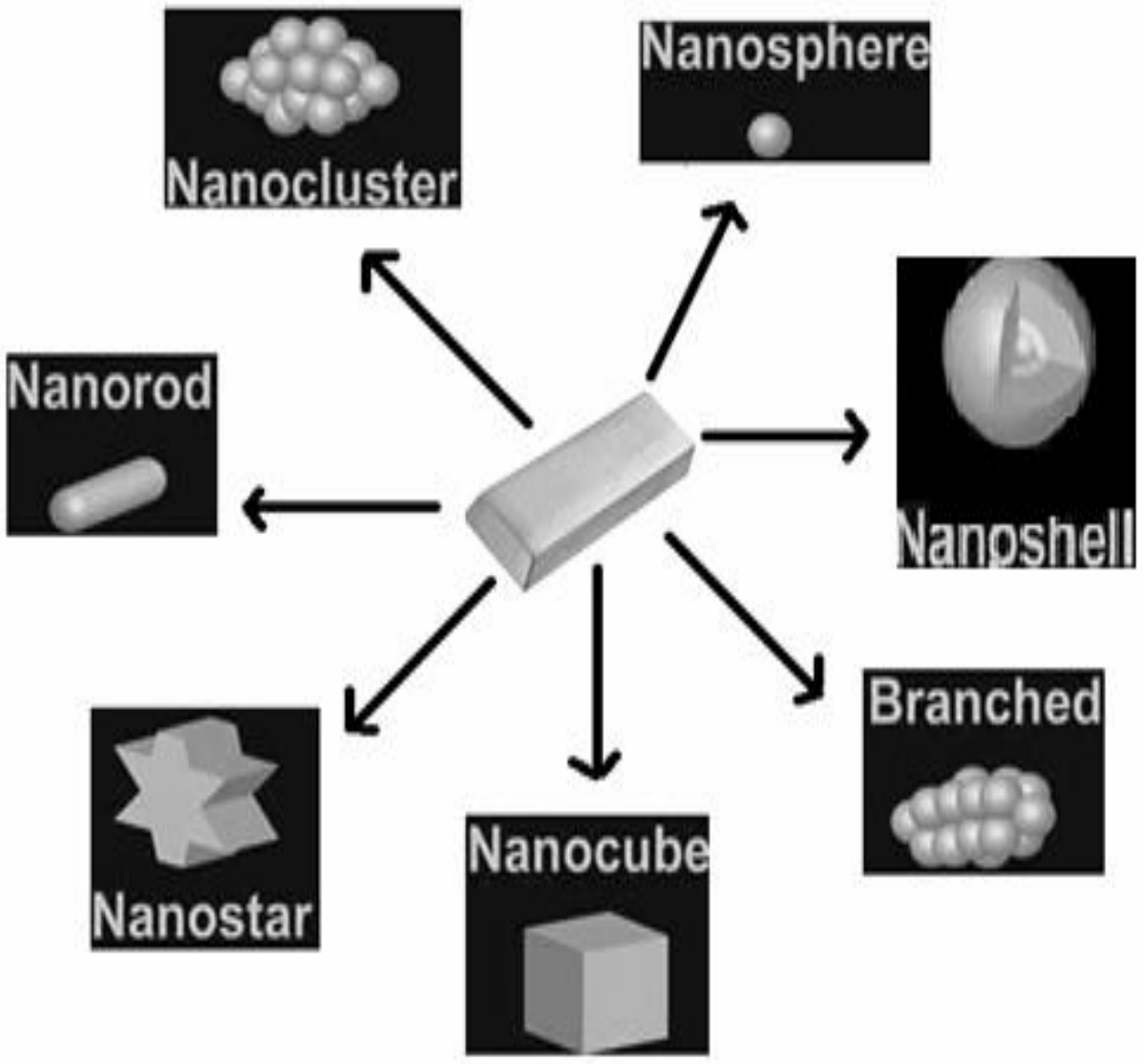


Figure 1.1: Various shapes of gold nanoparticles [8]

1.3 Gold nanoparticles

Properties of gold nanoparticles are different from its bulk form because bulk gold is yellow solid and it is inert in nature and used for jewelry [10]. As the noblest of all metals, gold is very stable (e.g. it does not react with oxygen or sulphur). And are reported to be anti-oxidant

However, if gold is shrunk to a nanoparticle, it changes color, becoming red if it is spherical (Figure 1.2) and even colorless if it is shaped in a ring. Moreover, gold nanoparticles become very reactive [11]

Gold nanoparticles have strong affinity for alkynes as compared to other transition metal catalysts but the homogeneous systems are not favorable economically and environmentally because of rapid reduction of active gold complexes in to inert metallic gold during the C-H alkynes' activation. Due to the unique optical and electronic properties of gold nanoparticles they have been widely used in the color indicating probes in the development of analytical techniques which are used for the sensing of various analytes [12].

Figure1.2: Gold colloid is ruby-red, not golden [11]

1.3.1 Properties of gold nanoparticles

Chemical properties: GNP is known for being generally inert and, especially gold, for not being attacked by O2 to a significant extent. This makes GNPs stable in ordinary conditions. and also reactive with sulphur [13].

Optical properties: GNP exhibit strong absorption of electromagnetic waves in the visible range due to Surface Plasmon Resonance (SPR). SPR is caused due to collective oscillations of the conduction electrons of nanoparticles upon irradiation with visible light. The SPR is highly influenced by shape and size of the nanoparticles [13].

Physical properties: Since solid to liquid transition begins at interfaces, a well-known feature of nanometric particles is the lower melting temperature with respect to the bulk.

For instance gold undergoes a decrease in melting temperature of about 400ºC going from 20 nm to 5 nm particles and about 50 ºC going from bulk to 20 nm particles [10]. Thermal conductivity is enhanced for small particles due to higher surface to volume ratio, while

phonons energy become higher for very small particles and Raman spectroscopy can be used to measure cluster Size [15].

Electrical Properties: nanoparticles are good conductors, which is why they are used in electronics and wiring. Metals are good conductors because their electrons are not bound to individual atoms instead forming a “cloud” around the atomic cores. This cloud of electrons is mobile allowing metal to transport charge (electrons) easily [16].

1.4 problem Statement

Failure to store and handle vaccines properly can reduce vaccine potency, resulting in inadequate immune responses in patients and poor protection against disease.

Patients lose confidence in vaccines and their providers when revaccination is necessary because the vaccines they received may have been compromised (exposed to inappropriate conditions/temperatures or handled improperly).

1.5 Objective

Introduce an indicator based on the use of gold nanoparticles that detect vaccines container temperatures change from freezing to unfreezing manners in stores and after transportation and Distributions.

Specific objectives

1. Biosynthesis GNPs; Get stabilized, biocompatible GNPs in a clean, nontoxic environmentally with low cost which it used in medical applications by using plant seeds extracts.
2. Characterize GNPs by using various techniques, which provide important information for the understanding of different physicochemical features of materials like UV-VIS spectrophotometer and transition electron microscope.
3. Use the synthesized material to study temperature changes from freezing to unfreezing manner and Use the study to perform vaccine container efficiency detector.
4. Test the indicator in vaccine stores (unites refrigerators and small cold container).
5. Marketing and offering the labels for customers in cheap price.

1.6 Methodology

The concept of the new bio analytical application for the detection of vaccine freezing temperatures' is based on the use of GNRs, located close to vaccines or eventually inside a

Container and refrigerators. In freezing case the GNRs bulk are colorless due to electron relaxation.

When the temperature changes the GNRs are converted from bulk to colloidal manner and will return to original gold color.

In the first part of this study will produce GNPs by using plant extracts as biosynthetic method. According to black cumin seeds (*Nigella sativa*) and fenugreek seeds (*Trigonella foenum-graecum)* as reducing agents for the reduction of gold salts to the corresponding gold nanoparticles. The GNPs generated through these plants-mediated processes were characterized to give information about size and shapes for making them very useful for biomedicine application.

In the second part, the work includes using of produced GNPs to develop vaccines container efficiency detector which sensitive to color change when the temperature change.

The method steps:

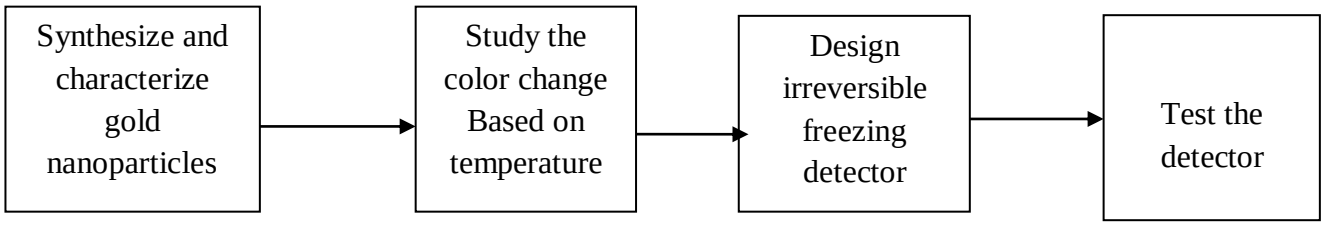


Figure 1.3: block diagram of label design

1.7 Thesis layout

This thesis consists of five chapters: chapter one includes project introduction, problem statement and project objectives. Then Chapter two is a Biosynthesis of Gold Nanoparticle by Fenugreek (*trigonella foenum*) and black Seed (*Nigella sativa*) extracts. And Chapter three is Characterization of gold Nanoparticles While Chapter four explain the Vaccines container detector based on gold nanoparticles. Finally Chapter five includes conclusion and recommendations.

CHAPTER TWO

Biosynthesis of Gold Nanoparticle

2.Biosynthesis of Gold Nanoparticle

2.1 introduction

Synthesis of metal nanoparticles is one of the most active and promising areas of research in nanotechnology because they display unique properties different from those of bulk metals due to their unique size and shape dependent characteristics [17].

2.2 Methods of the synthesis of nanoparticles

Nanoparticles are broadly classified in to two categories, Organic nanoparticles and inorganic nanoparticles. Organic nanoparticles include carbon nanoparticles and inorganic nanoparticles include metal nanoparticles (Ag, Au, Pt, and Pd), magnetic nanoparticles and semi-conductor nanoparticles (TiO2, SiO2, and ZnO2).

In general there are two processes used in the synthesis of nanoparticles: top-down process and bottom-up process. In top-down process bulk material is broken down into particles at nanoscale with different lithographic techniques such as grinding, milling etc, and in bottom-up approach, atoms self-accumulate to new nuclei which convert into a particle of nanoscale (figure 2.1) [18].

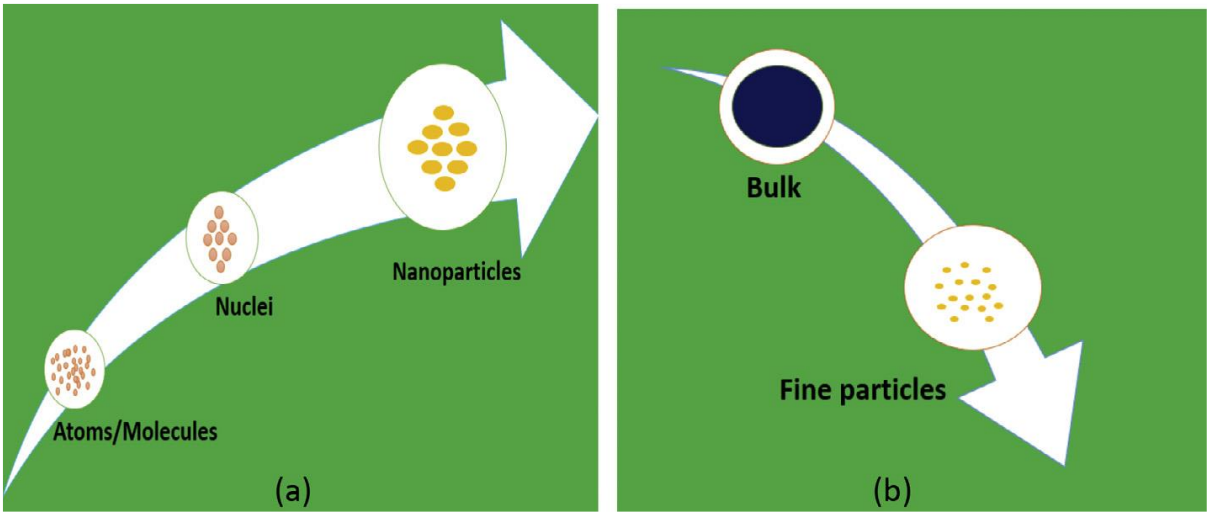


Figure 2.1: Protocols employed for synthesis of nanoparticles (a) bottom to top approach and (b) top to bottom approach [19]

Nanoparticles can be produced by either conventional physical and chemical methods or modern green (biological) synthesis.

2.2.1 Conventional methods

The conventional methods include ion sputtering, solvothermal synthesis, reduction and sol–gel technique. However, overall these methods are energy demanding, expensive, and are not eco-friendly. Due to the utilization of toxic chemicals and nonpolar solvents and later on synthetic additives or capping agents, their applications in clinical and biomedical fields are prohibited. Consequently, the need for the development of a clean, reliable, biocompatible, benign, and ecofriendly process to synthesize nanoparticles leads to turning researchers toward 'green' chemistry and bioprocesses [18].

The possibilities of employing plants in the deliberate synthesis of nanoparticles are attracting growing interest as an important source towards a reliable and environmentally benign method of metallic nanoparticles synthesis and its characterization (figure 2.2).

2.2.2 Green (biological) methods

The green (biological) methods of synthesizing nanoparticles using naturally occurring reagents such as vitamins, sugars, plant extracts, biodegradable polymers and microorganisms as reductants and capping agents are proven to be more environmental friendly and effective. Plant parts such as leaf, root, latex, seed, and stem are being used for metal nanoparticle synthesis. The key active agents in some of these syntheses are believed to be polyphenols present.

Greener synthesis of nanoparticles provides advancement over other methods as it is simple, cost-effective, and relatively reproducible and often results in more stable materials [13].

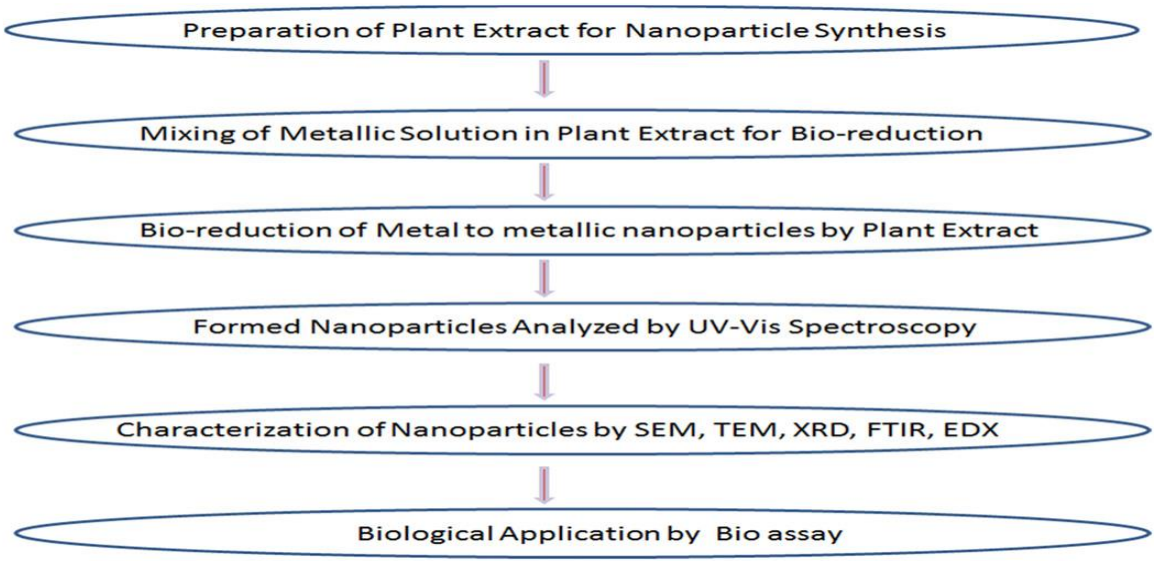


Figure 2.2: Steps involved in the synthesis of nanoparticles[18].

2.2.2.1 Plant seeds extract methods:

The plant extracts were prepared using unexplored two types of seeds: black seed (*Nigella sativa*) and fenugreek seed (*Trigonella foenum graecum*). Black seed, as shown in (figure 2.3a), is a member of the Ranonculaceae family and native to some parts of the mediterranean region. Recently, many medical properties have been attributed to the black cumin seeds, including antineoplastic (antitumour), antibacterial, antifungal, antihelmenthic and treatment of asthma. The seeds which used for culinary, as well as medical purposes, have been shown to contain high levels of antioxidants [20]. While fenugreek (*T. foenum-graecum*) seed as shown in (figure 2.3b) is an herb that is commonly found growing in the Mediterranean region of the world. While the seeds and leaves are primarily used as a culinary spice, it is also used to treat a variety of health problems in Egypt, Greece, Italy and South Asia. Fenugreek seeds have been found to contain protein, vitamin C, niacin, potassium, and diosgenin (which are a compound that has properties similar to estrogen). Other active constituents in fenugreek are alkaloids, lysine and L-tryptophan, as well as steroidal saponins (diosgenin, yamogenin, tigogenin, and neotigogenin) [21, 22]. Fenugreek has also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive and antioxidant activity [22].

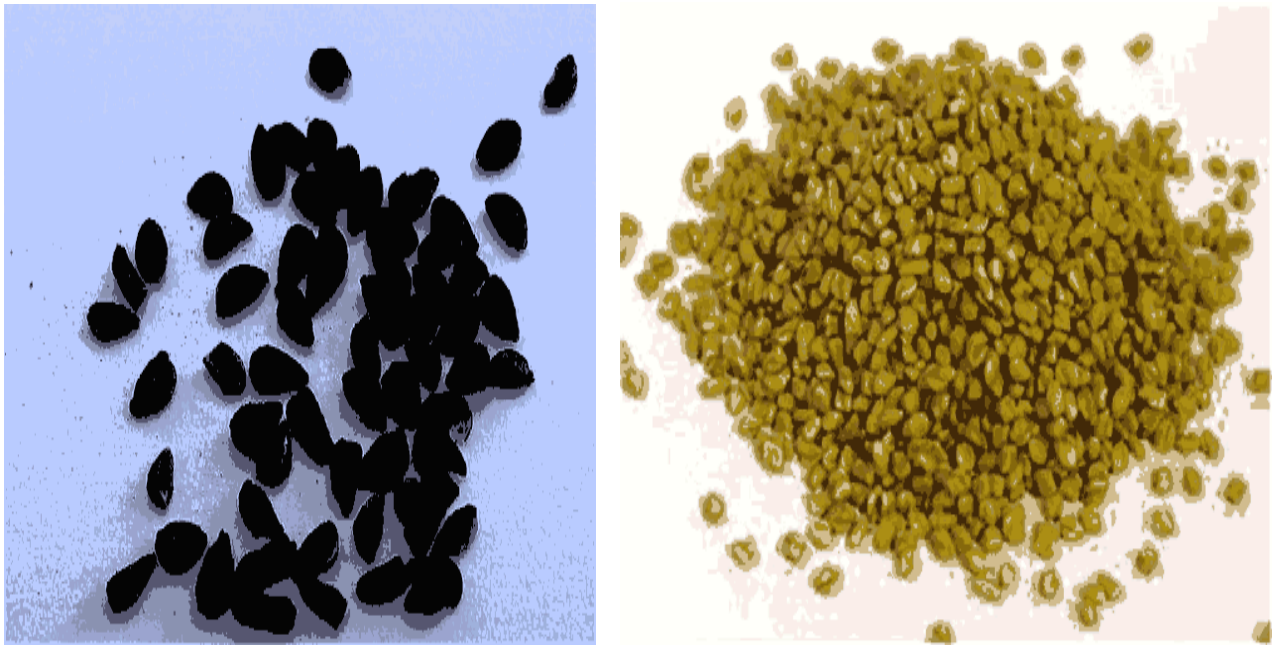


Figure 2.3: (a): Blak seed (*Nigella sativa*) (b): Fenugreek seed(*Trigonellafoenum graecum*)

2.3 Reviewof gold nanoparticles syntheses

2.3.1 Review of synthesis gold nanoparticles by plant

In 2014 in batra et al, they develop research about “Phytofabrication of nanoparticles through plant as nanofactories”.In recent years, nanoscience and nanotechnology have emerged as a new area of fundamental science and are receiving global attention due to their extensive applications. Conventionally nanoparticles were manufactured by physical and chemical techniques. The recent development and implementation of new technologies have led to a new trend, the nano-revolution unfolding the role of plants in bio- and green synthesis of nanoparticles which seems to have drawn a quite unequivocal attention to the synthesis of stable nanoparticles. Although nanoparticles can be synthesized through many conventional methods, biological route of the synthesis is more competent than the physical and chemical techniques. Biologically synthesized nanoparticles have enjoyed an upsurge of applications in various sectors. Hence, the present study envisions biosynthesis of nanoparticles from plants which are emerging as nanofactories. Hence, the present review summarizes the literature reported thus far and envisions plants as emerging sources of nanofactories along with applications, the mechanism behind phytosynthesis of nanoparticles and the mechanism of antibacterial action of nanoparticles [23].

2.3.2 Review of synthesis gold nanoparticles by Fenugreek seed

s.Aswathy etal developed new synthesis methods for monodispersed nanocrystals using cheap and nontoxic chemicals, environmentally benign solvents and renewable materials remains a challenge to the scientific community. Most of the current methods involve known protocols which may be potentially harmful to either environment or human health. Recent research has been focused on green synthesis methods to produce new nanomaterials, ecofriendly and safer with sustainable commercial viability. The present work reports the green synthesis of gold nanoparticles using the aqueous extract of fenugreek (Trigonella foenum-graecum) as reducing and protecting agent. The pathway is based on the reduction of AuCl_4 by the extract of fenugreek. This method is simple, efficient, economic and nontoxic. Gold nanoparticles having different sizes in the range from 15 to 25 nm could be obtained by controlling the synthesis parameters [24].

2.4 Experimental method

This work attempted to illustrate the process of synthesis of gold nanoparticles by using plant seed extract containing the two types of seeds: black seed (*Nigella sativa*) and fenugreek seed (*Trigonella foenum graecum*).

2.4.1 Materials for synthesis of GNPs:

The fenugreek, black seeds and the Gum Arabic powder were purchased from a local herbal shop in the Sudan. Hydrogen tetrachloroaurate tetrahydrate (HAuCl4.3H2O) purchased from lab course trading enterprise Co. Ltd. (sudan) and used without further purification.

2.4.2 The instrument

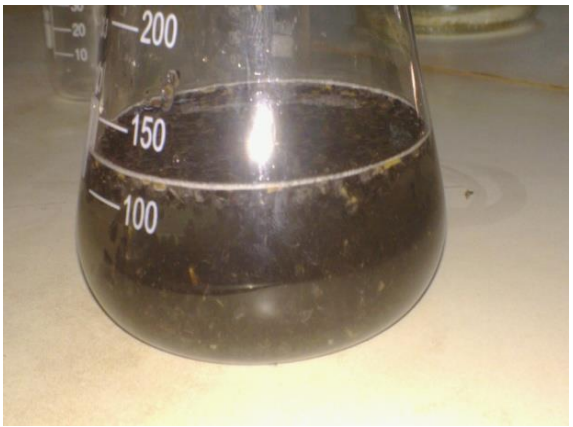
Sensitive balance to weighting seeds and Hydrogen tetrachloroaurate trihydrate (Gold salt) [KERN Scale], Centrifuge [centurion K_2 series] 8000 rpm, Microwave LG [MS3040S/00] 2450 MHz and Refrigerator at 4°c.

2.4.3 Bio synthise of GNP

2.4.3.1 Preparation of fenugreekand black Seeds Extract

The carefully weighted 8 g fenugreek and black seeds were washed with deionised water to remove any contaminant or dust particles. Fenugreek seeds were maintained for 24 h in 50 ml of deionised water at 25 º. for black seeds were maintained for 72 h, after the incubation period for tow of seeds, the supernatant was decanted and centrifuged 6000 rpm for 15 min at room temperature. Then it was stored at 4º in refrigerator. And used within 3 days for subsequent GNPs synthesis.

(a)



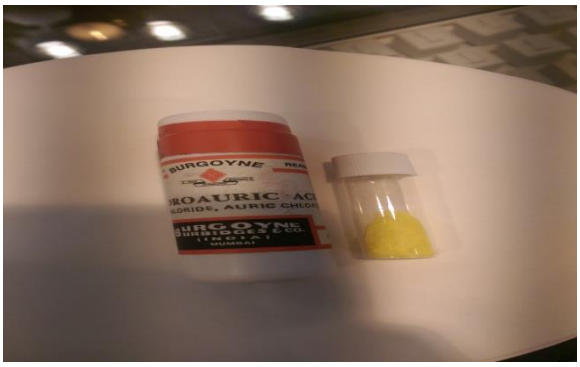
(b)

(c) (d)

Figure 2.4: (a) Black seeds (b) Black seeds extract (c) fenugreek seed (d) fenugreek seed extract

2.4.3.2 Preparation of Hydrogen tetrachloroaurate trihydrate solution:

Carefully weight one g of HAuCl4.3H2O powder using sensitive balance, added to 100 ml beaker and increase the volume to 29 ml with deionised water.



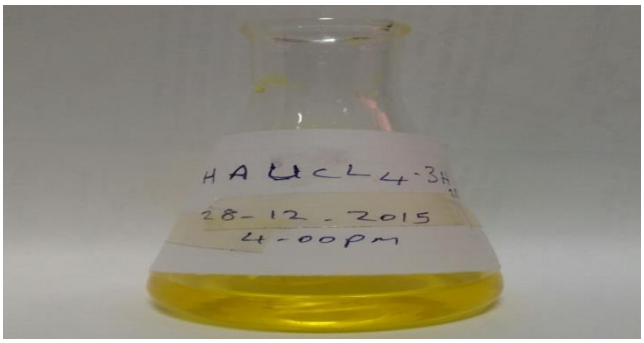


Figure 2.5: (a) HAuCl4.3H2O powder (b) HAuCl4.3H2O solution

2.4.3.3 Biosynthesis of GNPs by Microwave Irradiation

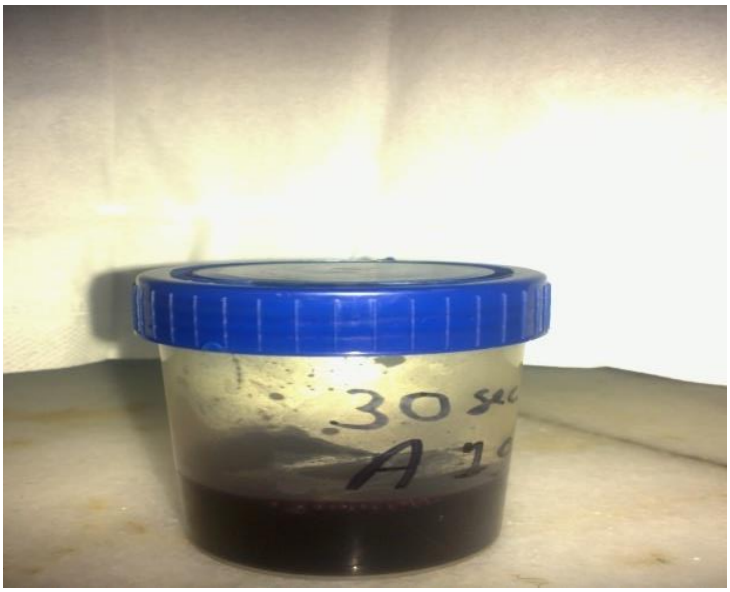
In a typical experiment, to 100 ml beaker was added 120 mg of gum Arabic powder, 10 ml of fenugreek, black seed extract and the volume increased to 20 ml by addition of an appropriate volume of deionised water. To the resulting mixture 16 ml aqueous solution of 0.1 mM [HAuCl4.4H2O] was immediately added as shown in figure (2.6 a). Following this, the beaker was placed in the centre of a domestic microwave oven (MS3040S/00) at 2450MHZ, 850W as shown in figure (2.6 b).

After just 30 s or 60 s of microwave irradiation, the color of the stirred mixture turned purple-red from pale yellow indicating the formation of GNPs. The solution was then left to

cool to room temperature and the rapid reduction is complete within 2 min by stable light purple- red color of the solution which gives 10 ml colloid. To obtain 8 and 6 ml colloids the addition of the fenugreek and black seed extract is varied as 8, 6 ml, respectively.

(a)

(b)



(c)

Figure 2.6: synthesized GNPs by microwave irradiation; (a) the resulting mixture, (b) synthesized GNPs after microwave irradiation, (c) Synthesized GNPs after microwave irradiation at 30s

2.4.4 Results and discussion

Depending on (figure 2.6 b and c) GNPs generated by reduction of gold precursor of Au^{+3}(HAuCl) to Au^{0}(HAuCl) by a reducing agent (bacterial biomass, plant seed extract) in the presence of a stabilizer (gum arabic) which keeps NPs apart, thus avoiding their aggregation.

After just 30 s of microwave irradiation, the color of the stirred mixture turned purple-red from pale yellow indicating the formation of GNPs

The colloidal gold is stable for long duration in absence of any special of stabilizing agent [23].

GNPs solutions were synthesized by fenugreek looked visibly the same after 4 months of synthesizing.

2.4.5 Conclusion

The green (biological) synthesis of gold nanoparticles using the plant extracts was prepared using two types of seeds: black seed (*Nigella sativa*) and fenugreek seed (*Trigonella foenum graecum*) as reducing and capping agents. This method is simple, efficient, economic and environmentally benign. Further, the as-prepared gold NPs show size-dependent catalytic activity.

This work visually describes each stages of GNPs synthesis from the preparation of black seed and fenugreek extracts with adding gold salt in presence of Gum Arabic to keeps NPs apart from aggregation , the color of resulting mixture of final solution turned purple-red from pale yellow indicating the formation of GNPs after using microwave irradiation method

Gum Arabic (GA) which belongs to the arabinogalactan-protein family is the oldest and best known of all the tree gum exudates. Today, this natural gum is widely used in the pharmaceutical and food industry as an emulsifier [25].

In the microwave method of synthesis, microwave radiations are introduced in the reaction solution. The microwave-assisted synthesis of nanoparticles has become popular due to its simplicity, ease of operation, rapid volumetric heating and kinetics, short reaction period and increasing yield of products compared to the conventional heating methods [26, 27]. Microwaves are a form of electromagnetic energy, with frequencies in the range of 300MHz to 300 GHz. The commonly used frequency is 2456 GHz

Several factors such as pH, temperature, concentration of plant extract, concentration of metal solution, incubation/ reaction time etc, affect the synthesis, size and shape of nanoparticles [23].

CHAPTER THREE

Characterization of gold Nanoparticles

3. Characterization of gold Nanoparticles

3.1 Introduction

To understand the control of synthesis and their applications, it is very important to characterize the nanoparticles. There are many different techniques available for the characterization of nanoparticles [28].

The characterization will give information about the absorption spectrum of the Plasmon band, Size, shape and the morphology of the gold nanoparticles.

3.2 Physicochemical characterization of nanomaterials

The nanomaterials can be characterized using various techniques, which provide important information for the understanding of different physicochemical features of materials. Some of the most extensively used techniques for characterization of Nanomaterial‟s are as follows [5]:

(a) Optical Spectroscopy

(i) Ultraviolet-visible (UV-Vis) spectroscopy.

(ii) Fourier transforms infrared (FTIR) spectroscopy.

(iii) Fluorescence spectroscopy.

(b) X-ray diffraction (XRD).

(c) Scanning electron microscopy (SEM).

(d) Transmission electron microscopy (TEM).

(e) Atomic force microscopy (AFM).

(f) Thermal Analysis (TA).

3.2.1 Optical Spectroscopy

Optical spectroscopy has been widely used for the characterization of nanomaterials and the techniques can be generally categorized into two groups: Ultraviolet-visible (UV-Vis) spectroscopy and emission (fluorescence) and vibration (infrared) spectroscopy.

The former determines the electronic structures of atoms, ions, molecules or crystals through exciting electrons from the ground to excited states (absorption) and relaxing from the excited to ground states (emission). The vibration technique involves the interactions of photons with species in a sample that results in energy transfer to or from the sample via vibrational excitation or de-excitation. The vibration frequencies provide the information of chemical bonds in the detecting samples [5].

3.2.1.1 UV-Vis Spectroscopy

It deals with the study of electronic transitions between orbitals or bands of atoms, ions or molecules in gaseous, liquid and solid state [29]. The metallic nanoparticles are known to exhibit different characteristic colors [30]. This absorption of electromagnetic radiation by metallic nanoparticles originates from the coherent oscillation of the valence band electrons induced by an interaction with the electromagnetic field [31].These resonances are known as surface Plasmon, which occur only in the case of nanoparticles and not in the case of bulk metallic particles [32].

Figure 3.1: UV-Vis Spectroscopy [uv-1800-SHIMADZU [33]

3.2.1.1.1 Color in metal colloids (surface Plasmon's)

One of the distinguishing properties of metal nanoparticles in general is their optical properties, which are different from those of their bulk counterpart. This is due to an effect called localized surface Plasmon resonance. In simple terms, when light hits a metal surface (of any size) some of the light wave propagates along the metal surface giving rise to a surface Plasmon a group of surface conduction electrons that propagate in a direction parallel to the metal/dielectric (or metal/vacuum) interface. When a Plasmon is generated in a conventional bulk metal, electrons can move freely in the material and no effect is registered. In the case of nanoparticles, the surface Plasmon is localized in space, so it oscillates back and forth in a synchronized way in a small space, and the effect is called Localized Surface Plasmon Resonance (LSPR). When the frequency of this oscillation is the same as the frequency of the light that it generated it (i.e. the incident light), the Plasmon is said to be in resonance with the incident light.

One of the consequences of the LSPR effect in metal nanoparticles is that they have very strong visible absorption due to the resonant coherent oscillation of the Plasmon. As a result, colloids of metal nanoparticles such as gold or silver can display colors which are not found

in their bulk form, such as red, purple or orange, depending on the shape, size and surrounding media of the nanoparticles [11].

3.2.1.2 Fourier Transform Infrared Spectroscopy

Fourier transforms infrared (FTIR) spectroscopy deals with the vibration of chemical bonds in a molecule at various frequencies depending on the elements and types of bonds. After absorbing electromagnetic radiation the frequency of vibration of a bond increases leading to transition between ground state and several excited states. These absorption frequencies represent excitations of vibrations of the chemical bonds and thus are specific to the type of bond and the group of atoms involved in the vibration. The energy corresponding to these frequencies correspond to the infrared region (4000–400 cm^{-1}) of the electromagnetic spectrum. The term Fourier transform (FT) refers to a recent development in the manner in which the data are collected and converted from an interference pattern to an infrared absorption spectrum that is like a molecular "fingerprint" [34]. The FTIR measurement can be utilized to study the presence of protein molecule in the solution, as the FTIR spectra in the 1400–1700 cm^{-1} region provides information about the presence of –CO- and –NH-groups [35].

Figure 3.2**:** FTIR spectroscopy [2400s-SHIMAZU] [36]

3.2.2 X-Ray Diffraction

X-ray diffraction is a very important technique that has long been used to determine the crystal structure of solids, including lattice constants and geometry, identification of unknown materials, orientation of single crystals, defects, etc. [37]. The X-ray diffraction patterns are obtained by measurement of the angles at which an X-ray beam is diffracted by the crystalline phases in the specimen. Bragg s (equation 3.1) relates the distance between

two (h, k, and l) planes (d) and the angle of diffraction (2θ) as: n λ = 2dsinθ, where, λ = wavelength of X-rays, n = an integer known as the order of reflection (h, k and l represent Miller indices of the respective planes) [38]. From the diffraction patterns, the uniqueness of nanocrystal structure, phase purity, degree of crystallinity and unit cell parameters of the nanocrystalline materials can be determined. X-ray diffraction technique is nondestructive and does not require elaborate sample preparation, which partly explains the wide use of XRD methods in material characterization.

X-ray diffraction broadening analysis has been widely used to determine the crystal size of nanoscale materials. The average size of the nanoparticles can be estimated using the Debye–Scherrer equation:

$$D = {}^{k\lambda}/_{\beta cos\theta} \qquad \text{equation (3.1)}$$

Where D = thickness of the nanocrystal, k is a constant, λ = wavelength of X-rays, β = width at half maxima of (111) reflection at Bragg‟s angle 2θ [39].

Figure 3.3: X-Ray Diffraction [lab-XRD600]

3.2.3 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) is one of the most widely used techniques for characterization of nanomaterials and nanostructures. The resolution of the SEM approaches a few nanometers, and the instruments can operate at magnifications that are easily adjusted from ~10 to over 300,000. This technique provides not only topographical information like optical microscopes do, but also information of chemical composition near the surface. A

scanning electron microscope can generate an electron beam scanning back and forth over a solid sample. The interaction between the beam and the sample produces different types of signals providing detailed information about the surface structure and morphology of the sample. When an electron from the beam encounters a nucleus in the sample, the resultant coulombic attraction leads to a deflection in the electron's path, known as Rutherford elastic scattering. A fraction of these electrons will be completely backscattered, reemerging from the incident surface of the sample. Since the scattering angle depends on the atomic number of the nucleus, the primary electrons arriving at a given detector position can be used to produce images containing topological and compositional information [40]. The high-energy incident electrons can also interact with the loosely bound conduction band electrons in the sample. However, the amount of energy given to these secondary electrons as a result of the interactions is small, and so they have a very limited range in the sample. Hence, only those secondary electrons that are produced within a very short distance from the surface are able to escape from the sample. As a result, high-resolution topographical images can be obtained in this detection mode [41].

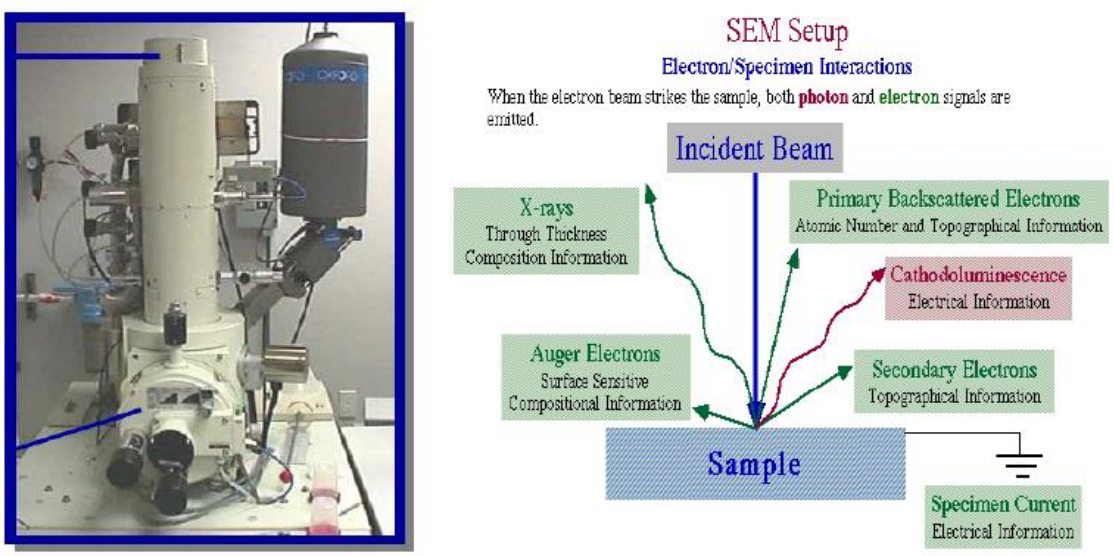


Figure3.4: Scanning Electron Microscopy [5]

3.2.4 Transmission Electron Microscopy

Transmission electron microscopy (TEM) is typically used for high resolution imaging of thin films of a solid sample for nanostructural and compositional analysis.

The technique involves: (i) irradiation of a very thin sample by a high-energy electron beam, which is diffracted by the lattices of a crystalline or semicrystalline material and propagated

along different directions, (ii) imaging and angular distribution analysis of the forward-scattered electrons (unlike SEM where backscattered electrons are detected), and (iii) energy analysis of the emitted X-rays [42] The topographic information obtained by TEM in the vicinity of atomic resolution can be utilized for structural characterization and identification of various phases of nanomaterials, viz., hexagonal, cubic or lamellar[45] One shortcoming of TEM is that the electron scattering information in a

TEM image originates from a three-dimensional sample, but is projected onto a two dimensional detector. Therefore, structural information along the electron beam direction is superimposed at the image plane. Selected area diffraction (SAD) offers a unique advantage to determine the crystal structure of individual nanomaterials, such as

nanocrystals and nanorods, and the crystal structures of different parts of the sample. In SAD, the condenser lens is defocused to produce parallel illumination at the specimen and a selected-area aperture is used to limit the diffracting volume. SAD patterns are often used to determine the Bravais lattices and lattice parameters of crystalline materials by the same procedure used in XRD [43].

In addition to the capability of structural characterization and chemical analyses, TEM has been also explored for the other applications in nanotechnology. Examples include the determination of melting points of nanocrystals, in which, an electron beam is used to heat up the nanocrystals and the melting points are determined by the disappearance of electron diffraction [44]. Another example is the measurement of mechanical and electrical properties of individual nanowires and nanotubes [45].

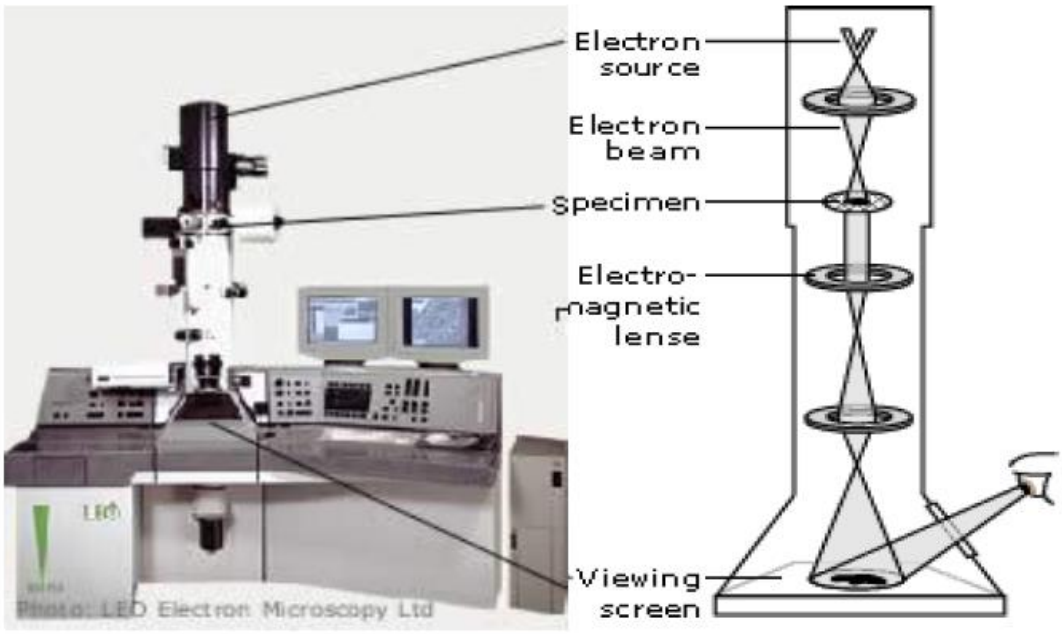


Figure3.5: transition Electron Microscopy [5]

Table 3.1: a summary of characterization techniques [28]

Technique	Measures	Sample	Sensitivity
TEM	particle size and characterization	Required<1mg sample Solid on substrate	down to 1 nm
SEM	particle size and characterization	conductive or sputter coated	down to 1 nm
AFM	particle size and characterization	Air or liquid	1 nm-8 μ m
X-ray diffraction (XRD) (>1mg) required	average particle size for a bulk sample	Arger crystalline samples	down to 1 nm
Fourier transform infrared spectroscopy (FTIR)	Substituent groups	Solid for ATR-IR or liquid	$20A^0$1 μ m

3.3 Review of gold nanoparticles characterization

3.3.1 Review of black seed characterization

Fregoon et al developed a research about "Biosynthesis of Controllable Size and Shape Gold Nanoparticles by Black Seed (Nigella Sativa) Extract". They found that the rapid and non-toxic method developed for the preparation of biocompatible gold nanoparticles by use of black seed (Nigella sativa) extract as antioxidant to treataqueous chloroauric acid solution by two different synthetic routes: microwave irradiation and thermo-induced procedures. The resulted nanoparticles were characterized and investigated by ultraviolet-visible (UV-Vis) spectrophotometry, transmission electron microscopy (TEM), energy-dispersive X-ray (EDX) spectroscopy, and X-ray diffraction (XRD). The size and shape of the nanoparticles were found to be very sensitive to the quantity of the extract also found that reaction temperature has a significant role in production of gold nanoparticles with different shapes. The XRD studies reflect an interesting feature indicates that gold nanocrystals are highly anisotropic in nature, mainly triangular and hexagonal shapes, and that the particles are (111) oriented. The observed characteristics suggest the application of the biocompatible gold nanoparticles to future in vivo imaging and therapy [17].

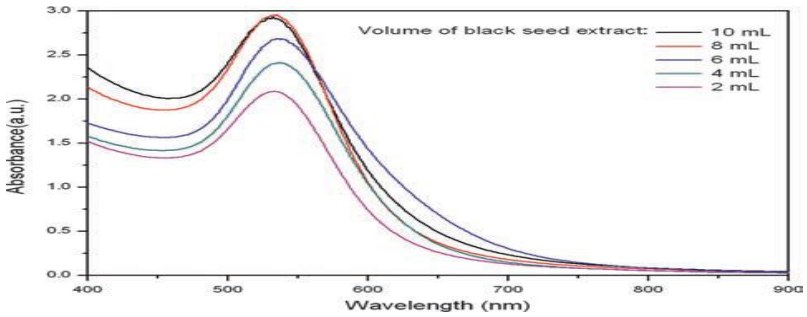


Figure 3.6: Absorption spectra of GNPs after bioreduction by black seed extract of 2, 4, 6, 8 and 10 ml dosages were exposed to 20 ml, 10 mM aqueous solution of HAuCl4 [17].

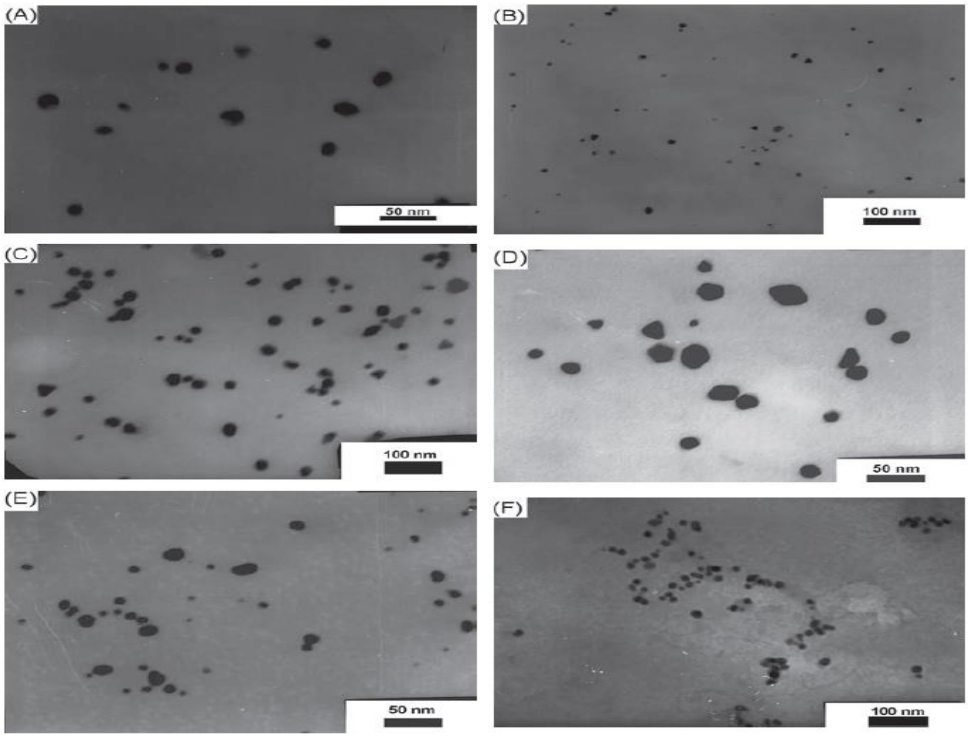


Figure3.7: TEM images illustrating the biosynthesis of GNPs using microwave irradiation by exposing (A) 4 ml, (B) 6 ml, (C) and (D) 8 ml, (E) and (F) 10 ml black seed extract to 20 ml, 10 mM aqueous HAuCl4. Scale bars: (A), (D) and (E) 50 nm; (B), (C) and (F) 100 nm [17].

3.3.2 Review of fenugreek characterization

Aswathy et al developed new synthesis methods for monodispersed nanocrystals using cheap and nontoxic chemicals, environmentally benign solvents and renewable materials remains a challenge to the scientific community. The nanoparticles have been characterized by UV–Visible spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD) and FTIR analysis. The high crystallinity of nanoparticles is evident from clear lattice fringes in the HRTEM images, bright circular spots in the SAED pattern and peaks in the XRD pattern. FTIR spectrum indicates the presence of different functional groups present in the biomolecule capping the nanoparticles. The synthesized gold nanoparticles show good catalytic activity for the reduction of 4-nitrophenol to 4-aminophenol by excess NaBH4. The catalytic activity is found to be size-dependent, the smaller nanoparticles showing faster activity [24].

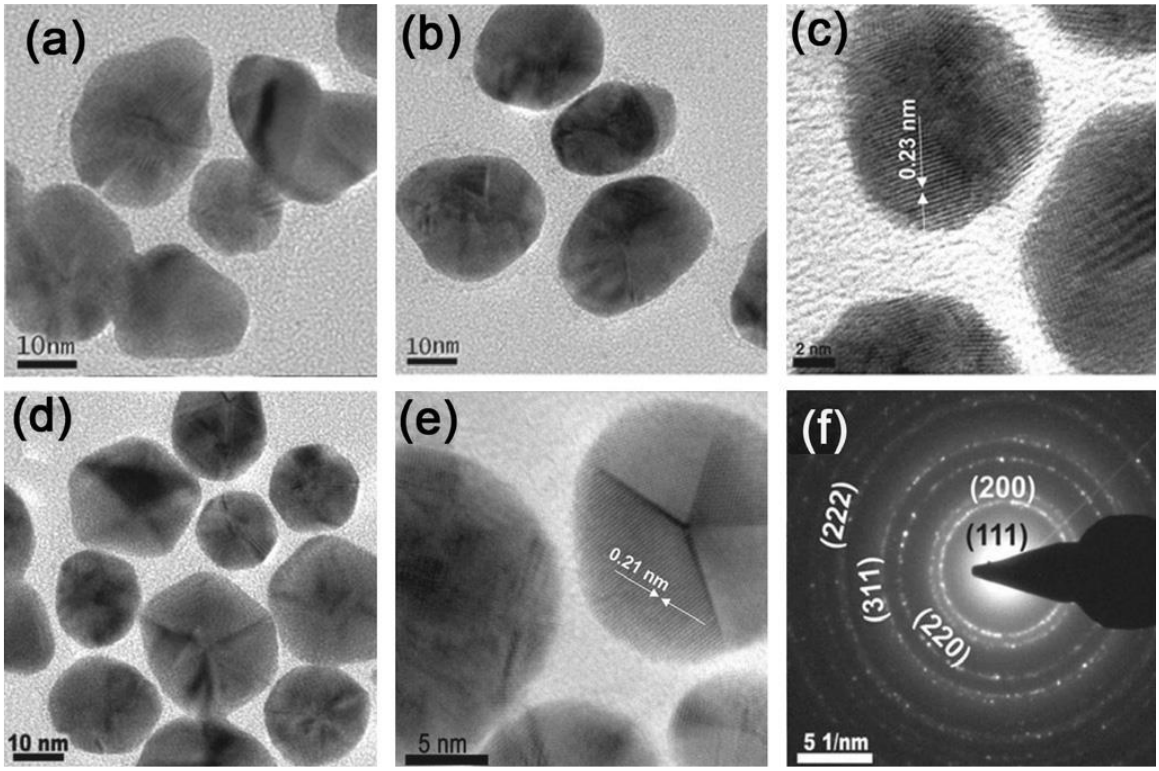


Figure 3.8: (a–c) TEM images of gold colloid g4 at different magnification and (d), (e) TEM images of gold colloid h4 at different magnification, (f) SAED pattern [24].

3.4 Experimental Method

3.4.1Materials

Three samples of GNPS were characterized: (1) black seed 10ml, (2) fenugreek 8ml and (3) fenugreek 10ml For TEM and XRD. And for UV – VIS spectroscopy all previous samples were characterized with adding fenugreek 6ml

3.4.2 Instrumentations

3.4.2.1 UV–VIS Absorption Spectroscopy:

Optical absorption spectra of the fenugreek seed extracted reduced GNPs were recorded using a UV-1800 UV–Vis-spectrophotometer (Shimadzu, Japan) with 2 ml of GNPs solution in a 1 cm optical path cuvette.

3.4.2.2 Fourier Transform Infrared Spectroscopy

3.4.2.3 Transition Electron Microscopy

The morphology of the GNPs was analyzed using the transition electron microscope (TEM) [JEOL-JEM-2100]

3.4.2.4 X-ray Diffractometer (XRD)

Resulting solutions of the developed GNPs were dried for the determination of the formation of Au by XRD [LAB-XRD600].

3.4.3 Result and Discussion

3.4.3.1 UV-Vis spectroscopy

3.4.3.1.1 black seed 10ml

Define Figure 3.9 exhibits UV-VIS spectroscopy curves for absorption of synthesized GNPs by black seed extract 10ml, the absorption at the wavelength range from 400nm – 700nm see (figure 3.6).

UV–VIS spectroscopy shows the appearance of the SPR band of 10ml at 561 nm.

Equation (3.1) can be used to calculate nanoparticles size (d) from the measurements of SPR wavelengths.

$$d = \frac{ln\left(\frac{\lambda spr - \lambda o)}{L1}\right)}{L2} \qquad \text{equation (3.2)}$$

λspr was the surface Plasmon resonance wavelength and d was the diameter. λ_0 = 512, L1= 6.53, and L2 = 0.0216 [46].

Table I shows the size of synthesized GNPs at different volume of fenugreek extract

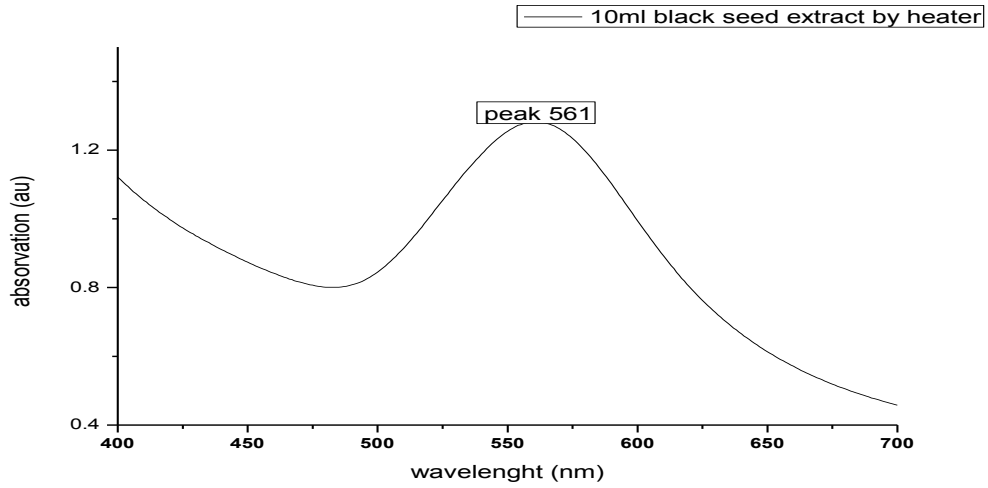


Figure 3.9: Absorption spectra of GNPs after bioreduction by black seed extract of 10 ml dosage was exposed to 20 ml, 10 mM aqueous solution of HAuCl4

3.4.3.1.2 Fenugreek seed UV-VIS Result

Define Figure 3.10 exhibits UV-VIS curves for absorption of synthesized GNPs by different amounts of the fenugreek seed extract, the absorption at the wavelength range from 400nm – 700nm.

UV–VIS spectra that were recorded at different dosages of extract for the reaction with the aqueous HAuCl4 show the appearance of the SPR band of 6 ml at 535.5 nm and 8ml at 534 nm while that of 10 ml at 535.6 nm.

This means that as the amount of fenugreek extract increased (6ml to 10ml) the peak shift to towards red or blue color .This is because the lowest quantities of the extract failed to protect most of the nascent nanoparticles from aggregating due to absence of sufficient of biomolecules act as protecting agents. In additional, this is responsible for the formation of the few particles.

Table 3.2: GNPs size determined by Equation 3.1

Volume of Fenugreek extract	Absorption	GNPs size [d]
6 ml	535.5nm	59.28nm
8ml	534.0nm	56.23nm
10ml	535.6nm	59.48nm

However, the equation (3.2) cannot be used for particles smaller than 25nm because the experimentally observed wavelength is lower than what would be expected. Recall that the surface Plasmon resonance (SPR) wavelength for spherical GNPs is usually around 540 nm and this experiment had a range of 534-535.6 nm. However, when particles were smaller than 25 nm, the wavelength of SPR was smaller than 520 nm. The wavelength may be smaller for particles smaller than 25 nm because of the increase of the ratio of surface atoms to bulk atoms for small particle diameters.

The position of SPR band in UV–Visible spectra is sensitive to particle size, shape, local refractive index and its interaction with medium. The amount of the black seed extract was found to be an important parameter in size disparity of GNPs.

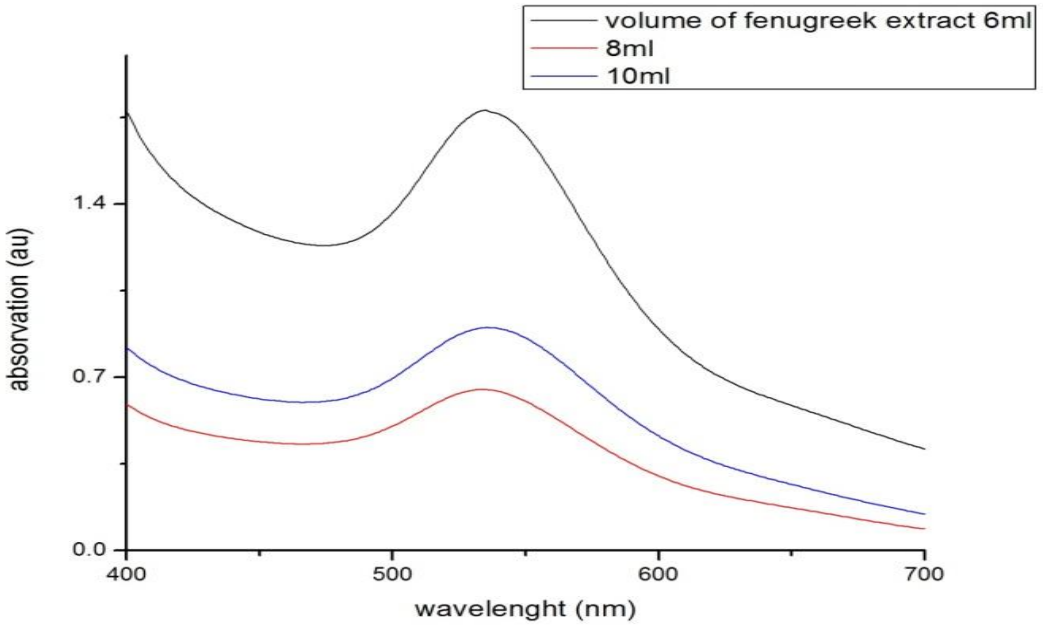


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Figure 3.10: Absorption spectra of GNPs after bioreduction by fenugreek seed extract of 6, 8 and 10 ml dosages were exposed to 20 ml, 10 mM aqueous solution of HAuCl4

3.4.3.2 Fourier Transform Infrared Spectroscopy (FTIR) result

FTIR measurements were carried out to identify the possible biomolecules present in fenugreek seed extract which are responsible for the reduction and capping of gold NPs. The spectrum (Figure 3.11) shows bands at (3454.24, 2084.91, 1647.10, 495.67, 472.53, 457.10 and 441.67) cm^{-1}.

The IR band due to O–H stretch, H–bonded is observed at 3454.24cm^{-1} it's strong and board absorption is identified as the alcohols, phenols.

The band located at 1647.10 cm^{-1} is due to the C=C tretching vibrations, is assigned as amid alkenes [47].

The bands (2084.91, 495.67, 472.53, 457.10 and 441.67) cm^{-1} may be assigned to the in plane and out of plane bending for benzene ring [48]. It is well-known that proteins can bind to gold NPs through free carboxylate group [49]. The presence of bands at 3454.24, 2084.91, 1647 cm^{-1} indicates that gold NPs are possibly bound to proteins through carboxylate group.

The phytochemical analysis of the dried seed extract of fenugreek has been reported to show the presence of proteins, vitamins, flavonoids, terpenoids, carotenoids, cumarins, curcumins, lignin, saponin and plant sterol [50]. The flavonoids present in the seed extract are powerful reducing agents which may be responsible for the reduction of chloroauric acid. The

carboxylate group present in proteins can act as surfactant to attach on the surface of gold NPs and it stabilizes gold NPs through electrostatic stabilization. Thus it is found that fenugreek seed extract has the ability to perform dual functions of reduction and stabilization of gold NPs.

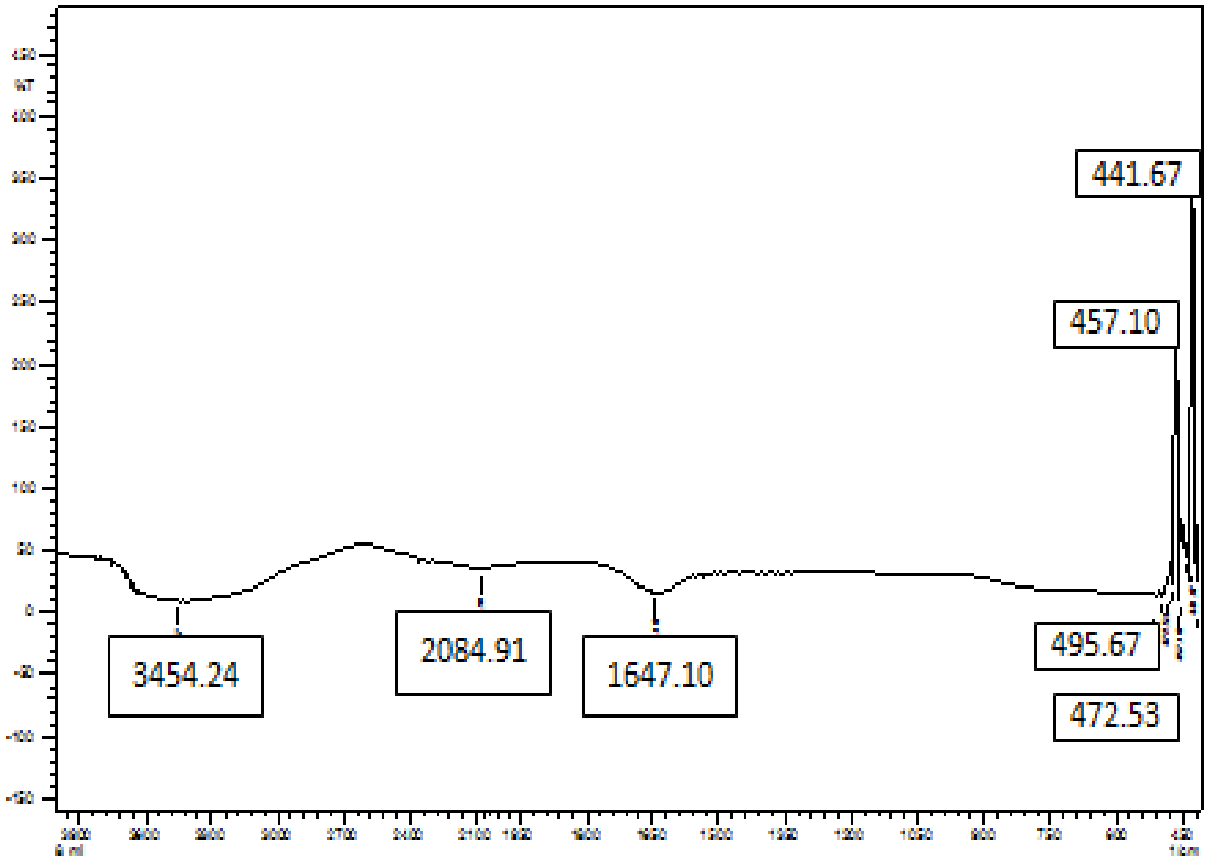


Figure 3.11: FTIR spectrum of gold nanoparticles. The inset shows the possible mechanism of formation of gold nanoparticles.

3.4.3.3 Transition Electron Microscopy result:

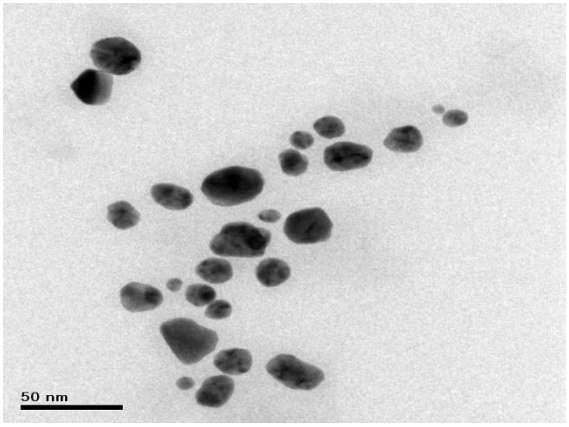
The size and morphology of the biosynthesized nanoparticles using black seed and fenugreek were characterized.

Typical TEM images obtained for 10 ml black seed colloids showed a uniform distribution and confirmed their spherical morphology figure (3.12) and mostly ranging from 6 to 19 nm in size.

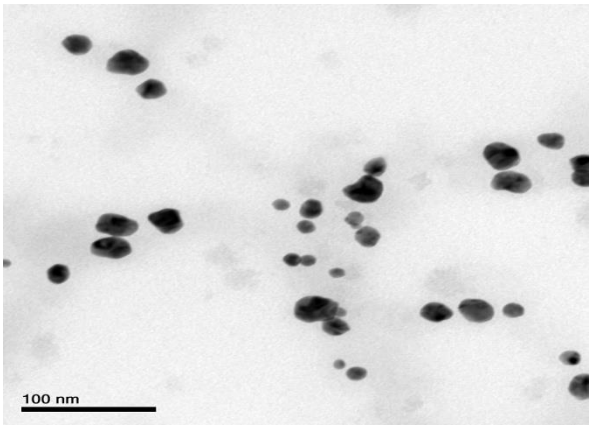
For fenugreek colloids images showed different shapes of GNPS like spherical, triangular, hexagonal, prisms and rod-shaped.

When the extract increases from 8ml to 10 ml dosage, the interaction was increased, leading to size reduction of the nanoparticles.

These results are in agreement with the shape of the SPR bands (Fig3.9and 3.10.) , as the dosage of fenugreek extract increased the stronger the interaction between bimolecular and nascent GNPs. Altering the size causes the GNPs to have different properties that are suitable for utilizations in biomedicine. Therefore, the prepared GNPs are suited for many potential biomedical applications.



(a)



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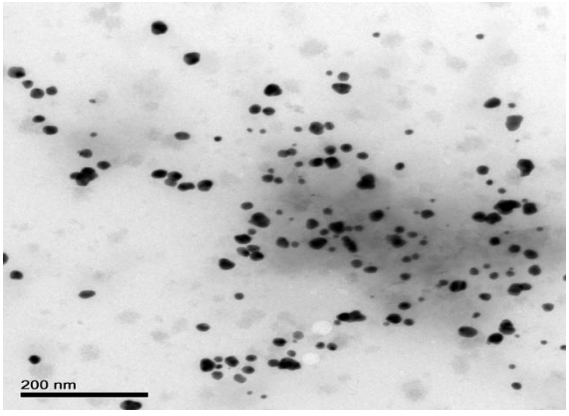

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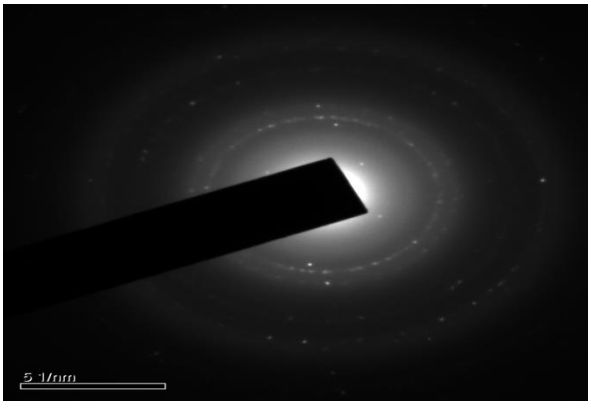

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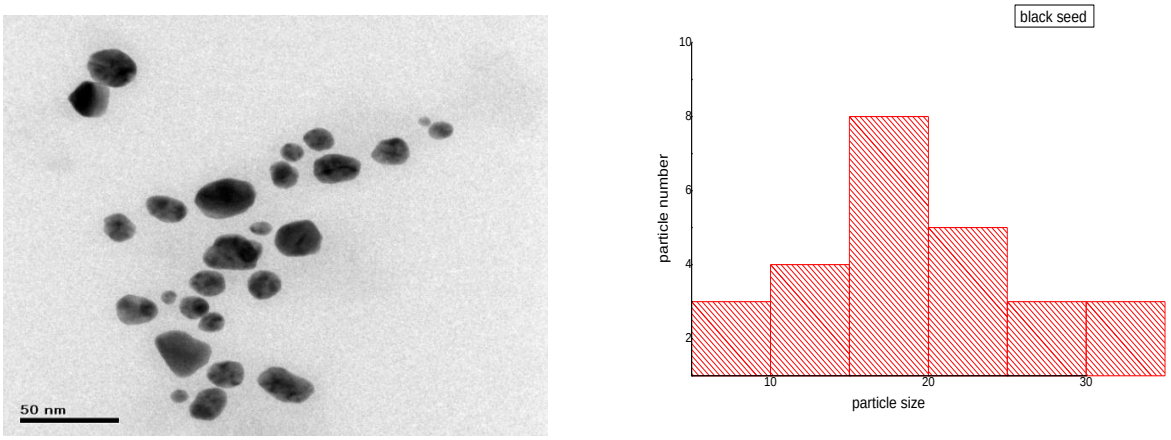
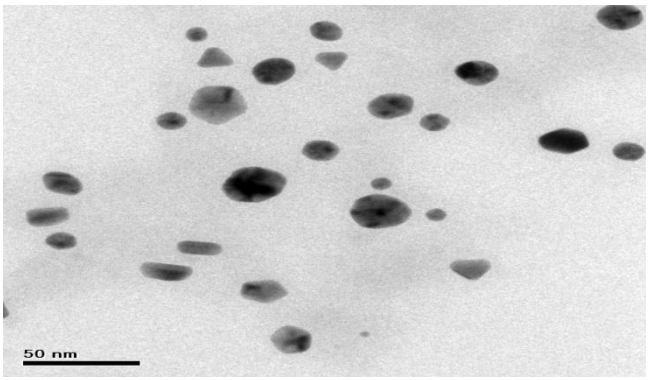
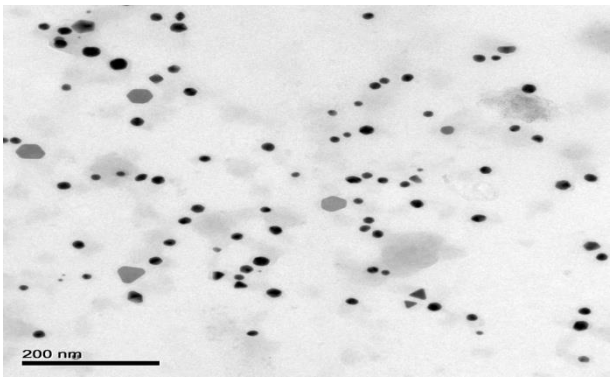


(e)

Figure 3.12 : (a–c) TEM images of black seed 10ml gold colloid at different magnification ,(d) SAED and (e) histogram of particle size number for corresponding image at 50 nm



(a)



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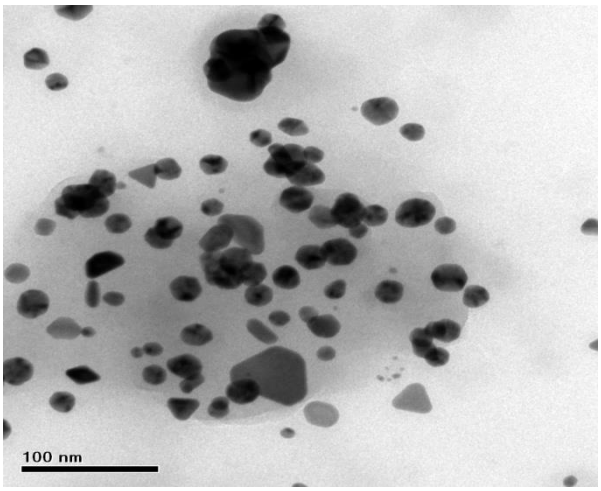

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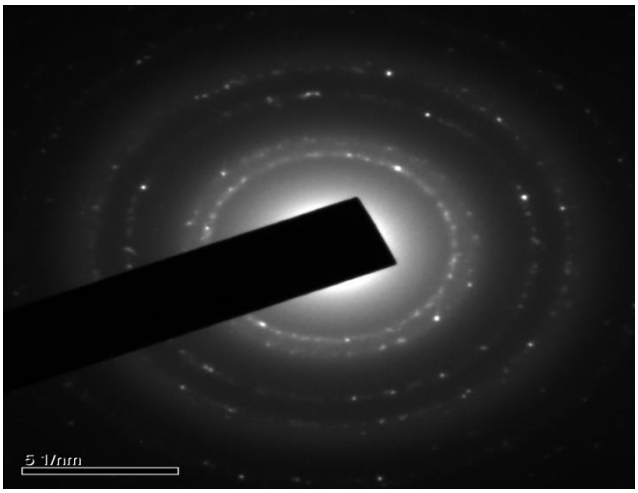

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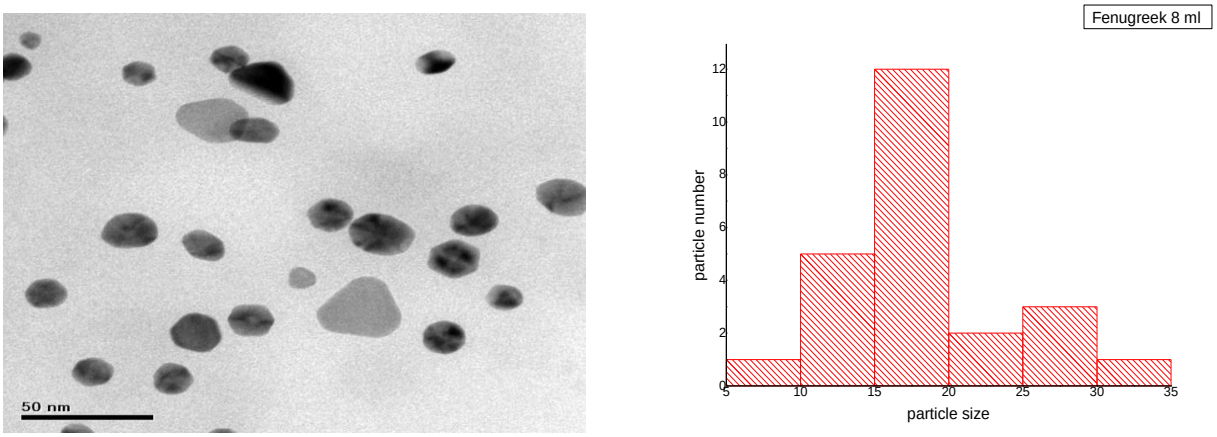
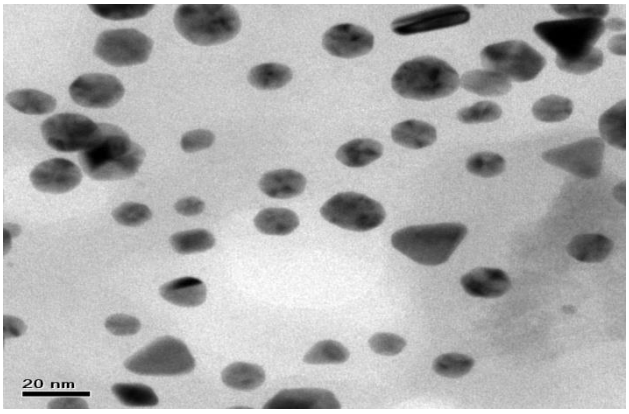
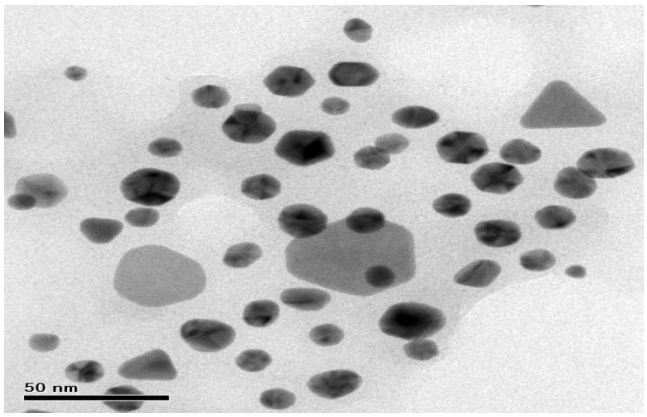


(e)

Figure 3.13 : (a–c) TEM images of fenugreek seed 8ml gold colloid at different magnification ,(d)SAED and (e) histogram of particle size number for corresponding image at 50 nm



(a)



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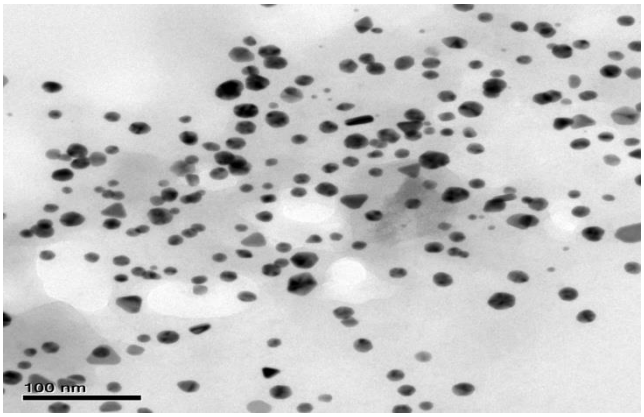

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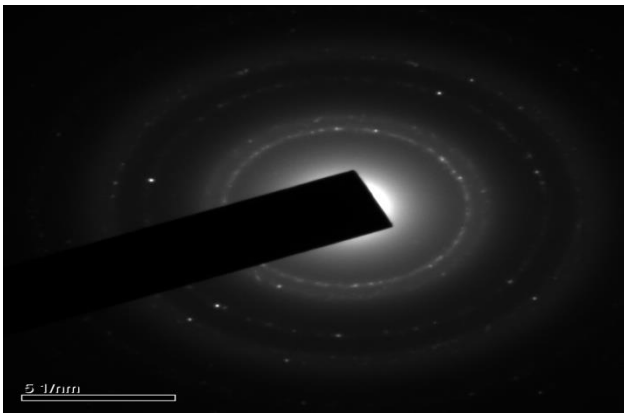

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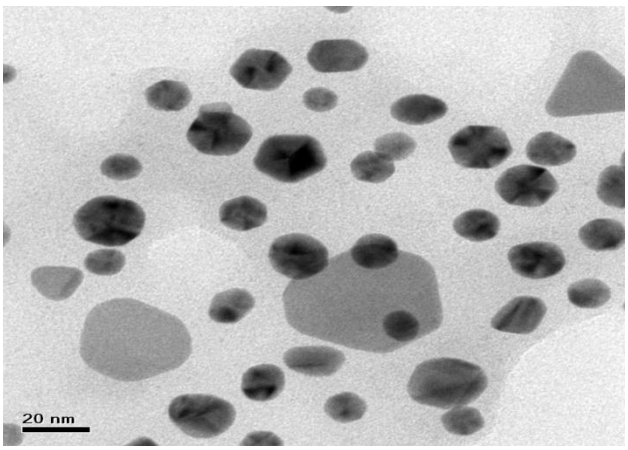
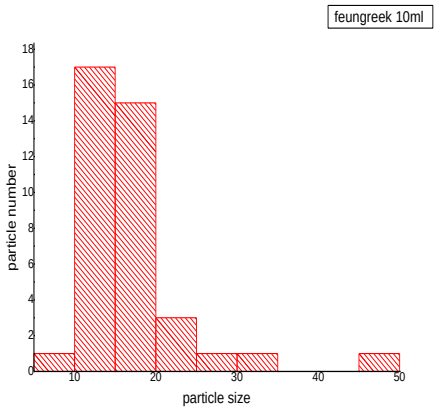




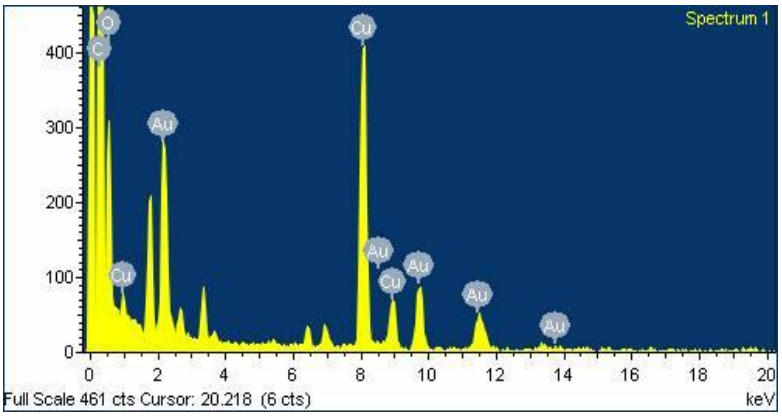
(e)

Figure 3.14: (a–c) TEM images of fenugreek seed 10ml gold colloid at different magnification, (d) SAED and (e) histogram of particle size number for corresponding image at 50 nm

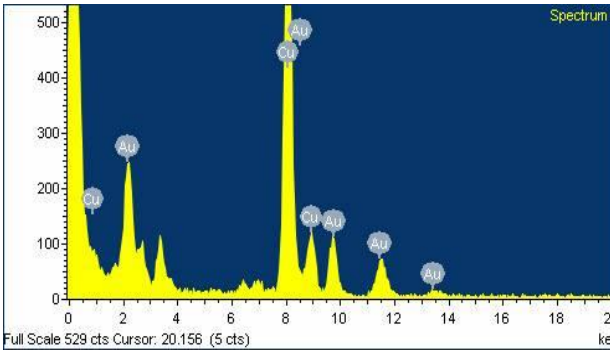
Energy-Dispersive X-ray (EDX) Spectroscopy

In the EDX spectrum of the GNPs, TEM imaging and the corresponding EDX analysis shown in figure 3.15 confirms the presence of Au in solution.

Copper peaks were also visible in the EDX spectra which were due to the Cu support grid. The lack of other elemental peaks and high amount of Au in the spectra confirms the purity of the gold in the transformed product. The presence of carbon and oxygen spots in the spectrum of black seed confirms the presence of stabilizers composed of alkyl chains



(a)



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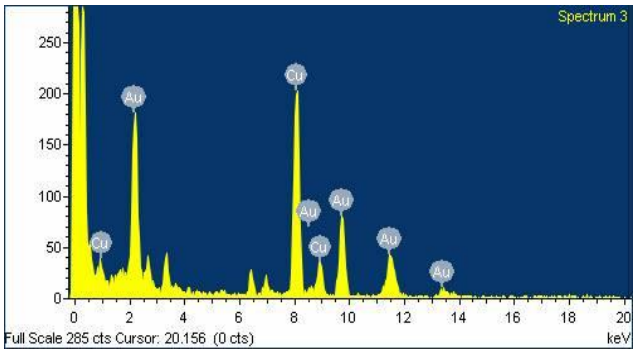


(c)

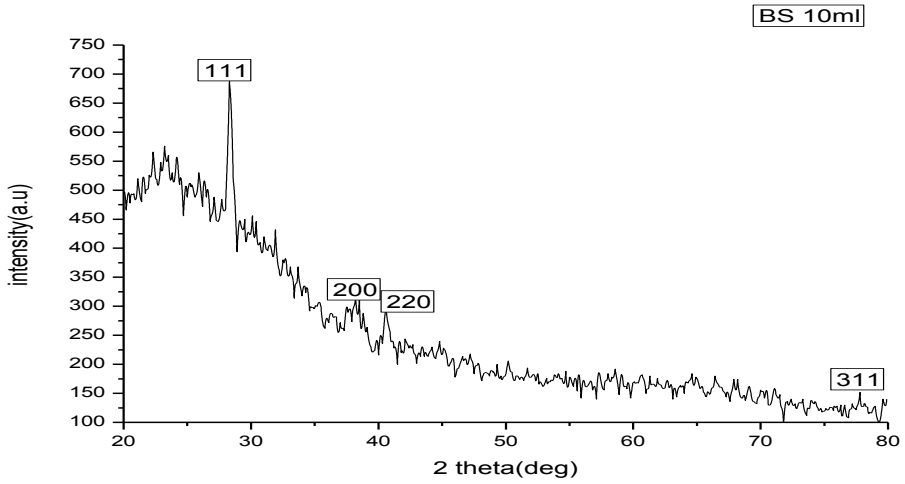
Figure 3.15: EDX spectrum of GNPs samples (a) black seed 10ml, (b) fenugreek 8ml and (c) Fenugreek 10ml.

3.4.3.4 XRD result:

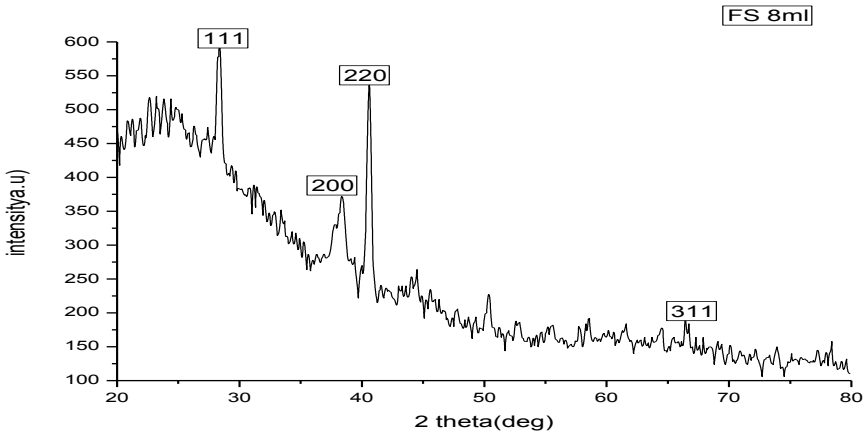
As apparent from the figure,

Figure 3.16 shows the XRD pattern of dried gold nanoparticles. The XRD peaks are found to be broad indicating the formation of nanoparticles. there is a broad peak that appeared at 2θ = 20° which can be attributed with 2θ values of 38°, 44°, 64.6°, and 77°. These bands

correspond to the 111, 200, 220, and 311 sets of lattice planes, which may be indexed as the bands for face centred cubic structures of Au. The XRD pattern, thus, clearly demonstrates that the Au NPs synthesized by the present green method are crystalline in nature. For black seed the peak corresponding to 111 plane is more intense than the other planes suggesting that 111 is the predominant orientation as confirmed by the high resolution TEM measurement.



(a)



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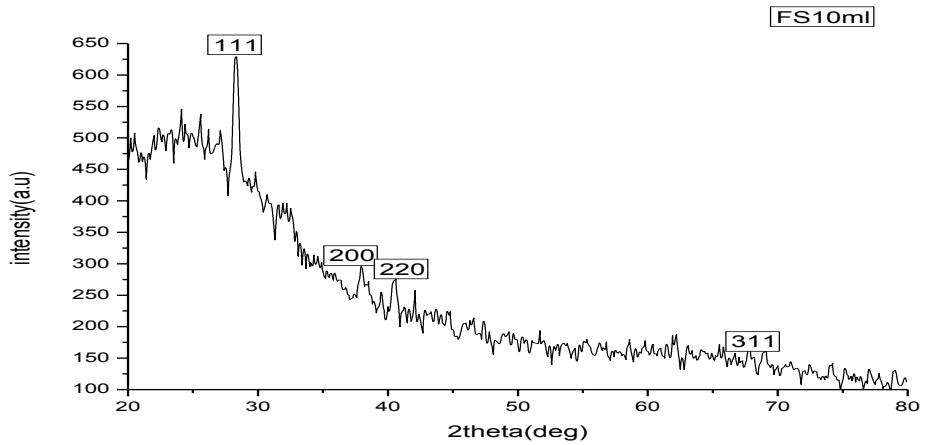


(c)

Figure 3.16: XRD pattern of gold nanoparticles (a) black seed 10ml, (b) fenugreek 8ml and (c) fenugreek 10ml.

3.4.4. Conclusion

Colloidal gold nanoparticles were synthesized according to the plant extract method and characterized by UV-VIS absorption spectroscopy, FTIR, transmission electron microscopy and X-ray diffraction. It was found that the concentration of the precursors affects the size of the nanoparticles. The result finding the Typical TEM images obtained for 10 ml black seed colloids consist of almost uniformly sized spherical nanoparticles, while fenugreek consist of different shapes of GNPS.

The particle diameters can be determined through experimental techniques. The best technique to use depends on the size of the particles. For example Equation (3.1) from UV-Vis spectroscopy can be used to calculate diameters of the particles when the absorbance ratio is known.

Since GNPs can form numerous shapes; such as prisms and rods, can determining the Size and Shape of Gold equation to calculate the diameters of these particles can be developed. Another idea to focus on for further research could be which shape is better for different applications. For example, each shape of GNPs have different physical properties thus making it useful to determine which shape is better in areas such as diagnostics, therapeutics, catalysis, optical sensing, and in further nanotechnology. Thus, a study could be conducted to learn how shape affects the application GNPs used [46].

CHAPTER FOUR

Vaccines detector based on goldnanoparticles

4.Vaccines detector based on goldnanoparticles

4.1 Introduction

Applications of nanoparticles in diagnosis, treatment, and monitoring of biological systems are slowly coalescing into a new field, often referred to as 'nanomedicine'[51]. Materials with nanoscale dimensions are of great interest in biomedical applications because their size is comparable to, or smaller than, that of many important biological entities such as genes (2 nm wide and 10–100 nm long), proteins (5–50 nm), viruses (20–450 nm), or cells (10–100 μm) [52]. These tiny particles can access otherwise unreachable regions of the organism and engage in interactions at molecular level or deliver a therapeutic load. For these reasons, it is widely accepted that systems incorporating either inorganic or organic nanoparticles have the potential to change dramatically the landscape of the biomedical field [53].

Due to their unique physical and chemical properties, gold nanoparticles are poised to play an important role in this exciting and dynamic field.

4.2 Properties of Colloidal Gold Relevant for Biomedical Applications

The unique properties of gold nanoparticles exploited in the bio-medical field depend on the size, shape, morphology, surface chemistry, and electrical charge. The ability to tailor these features as well as the biocompatibility of colloidal gold is central to all biomedical applications [54].

The various properties of different nanoparticles relative to bulk metals are summarized below.

Optical function: The surface absorption plasmon of Au can express various colors by changing the size of the particle, the form or shape of the particle, and the rate of condensation. A new paint that has the durability of an inorganic pigment and the vivid color of an organic substrate can be made. Nanoparticles smaller than the wavelength of light can be used to make high penetration conductivity materials (there is little absorption, dispersion, and reflection).

Catalyst function: Reaction efficiencies can be enhanced since the specific surface area of such nanoparticles is large compared with existing particles; to the extent that the surface terrace is regular at the atomic level, a hyperactive catalyst with high selectivity can be made: for example, Au nanoparticles.

Thermal function: When the particle diameter is small (less than 10 nm), the melting point is also lower than a bulk metal. Electronic wiring can be made with nanoparticles that have a low boiling point, for example, a polymer.

Electrical function: Since superconductivity transition temperature rises so that particle diameter is small (less than 1 nm), it can be used to make high temperature superconductivity material.

Mechanical function: Since the mechanical characteristics improve, mechanical strength can be sharply raised by mixing the nanoparticles with metals or ceramics.

Magnetic function: The attractive force of a magnetic metal increases on reduction of the particle diameter, such that soft-magnetic materials can be made in the form of an alloy of nanoparticles. Moreover, a permanent magnet can be made if the nanoparticles are smaller than the magnetic domain made to magnetize [55].

Surface Functionalization and Biocompatibility: The applied coating makes the nanoparticles biocompatible and imparts colloidal stability in both water and physiological media. In addition, modification of the particle surface by suitable (bio)molecules provides desired characteristics for the intended applications [51, 56, 57].

4.3 Biological and Medical Applications of Colloidal Gold

Nanotechnology is producing short-term impacts in the areas of:[58]

- Medical diagnostic tools and sensors
- Drug delivery
- Catalysts (many applications in chemistry and pharmaceuticals**)**
- Alloys (e.g., steel and materials used in prosthetics)
- Improved and body-friendly implants
- Biosensors and chemical sensors
- Bioanalysis tools
- Bioseparation technologies
- Medical imaging
- Filters

4.4Vaccines container efficiency detector

A particular example of gold nanoparticle application under study of vaccines quality and potency, Because of the extremely strong optical absorption of gold colloids, this colorimetric method is sensitive enough to be able to detect freezing manner change.

This analysis highlights that exposure of vaccines to freezing temperatures is pervasive, as well as within both the storage and transport segments of the cold chain.

4.4.1Value of Vaccine Storage and Handling Best Practices

Failure to store and handle vaccines properly can reduce vaccine potency, resulting in inadequate immune responses in patients and poor protection against disease. Patients lose confidence in vaccines and their providers when revaccination is necessary because the vaccine(s) they received may have been compromised (exposed to inappropriate conditions/ temperatures or handled improperly).Storage and handling errors can also result in significant financial loss if the vaccine cannot be used [59].

4.4.2 What is the Vaccine Cold Chain?

The vaccine cold chain is a temperature-controlled environment used to maintain and distribute vaccines in optimal condition. The cold chain relies on three main elements:

- Well-trained personnel
- Reliable transportation and storage equipment
- Efficient management procedures

The cold chain begins with the cold storage unit at the manufacturing plant, extends through transport of vaccine(s) to the distributor, then delivery and storage at the provider facility, and ends with administration of vaccine to the patient. Appropriate storage conditions must be maintained at every link in the cold chain [59].

4.4.3 Vaccine and Diluent Storage Temperatures

Freezer Temperature

Store frozen vaccines (e.g., varicella-containing vaccines [VAR, HZV, and MMRV]) in a freezer between -58°F and +5°F (-50°C and -15°C) until reconstitution and administration. These vaccines can deteriorate rapidly after removal from the freezer. Measles, mumps, and rubella vaccine (MMR) can be stored in a refrigerator or in a freezer.

Refrigerator Temperature

Store all other routinely recommended vaccines in a refrigerator between 35°F and 46°F (2°C and 8°C), with a desired average temperature of 40°F (5°C).This will allow for slight temperature fluctuations while still maintaining the recommended temperature range.

Diluents

Some diluents must be stored in the refrigerator. Other diluents have an option of being stored at room temperature (no warmer than 77°F [25°C]) or in the refrigerator [59].

4.4.4 Vaccine Potency

Excessive heat, cold, or light exposure can damage vaccines, resulting in reduced potency. Once potency is lost, it cannot be restored. Each time vaccines are exposed to improper conditions, potency is reduced further. Eventually, if the cold chain is not properly maintained, potency will be lost, and the vaccines become useless.

While exposure to any inappropriate conditions can affect potency of refrigerated vaccines, a single exposure to freezing temperatures will destroy some. Liquid vaccines that contain an aluminum adjuvant can permanently lose potency when exposed to freezing temperatures. Monitor the temperature of your storage unit(s) regularly [59].

4.4.5 Vaccine Appearance after Exposure to Inappropriate Storage Conditions

Some vaccines may show physical evidence that potency has been reduced when exposed to inappropriate storage conditions. This may appear as clumping in the solution that does not go away when the vial is shaken .Other vaccines may look normal when exposed to inappropriate storage conditions .For example, inactivated vaccines exposed to freezing temperatures (i.e., 32°F [0°C] or colder) may not appear frozen and give no indication of reduced or lost potency like Adacel, Boostrix, Cervarix, Comvax, Daptacel, Decavac. Vaccine appearance is not a reliable indicator that vaccines have been stored under appropriate conditions. Figure [59].

4.4.5 Review paper of Vaccines container efficiency detector

In 2008 Fredy Kurniawan aus Surabaya, Indonesia im März, prepared Freezing indicator from Gold nanoparticles which can change its color irreversibly when the solution become frozen (0^o C± 0.5), is one of the interesting property This property is used for the development of freezing indicator. This indicator may be useful for specific application. An attempt to stabilize nanoparticles has been performed by adding some additives. It is expected that the additives will give longer storage time or faster respond to temperature change. The list of the additives used can be seen in the table 4.1[60].

Table 4.1: the additives [60]

NO	Name of additives
1	Sodium cellulose phosphate
2	Zinc Dust
3	Silica Gel
4	Silver Iodide (home made, without purification)
4	Glucose
6	Snowmax 20µg/ml
7	Snowmax 80µg/ml
8	Snowmax 100µg/ml
9	No additive

The result of the test demonstrates at figure (4.2). It shows that zinc dust, silica gel, silver iodide (homemade, without purification) affect instability of the gold nanoparticles solution. The color of gold nanoparticles changes after addition of the additives in room temperature (Fig. 4.1a). Snowmax 100 µg/ml is considered to be the one of the fast additives that can change color (Fig 4.1b). After the gold nanoparticles is frozen completely, all the solution become colorless (Fig 4.1c)

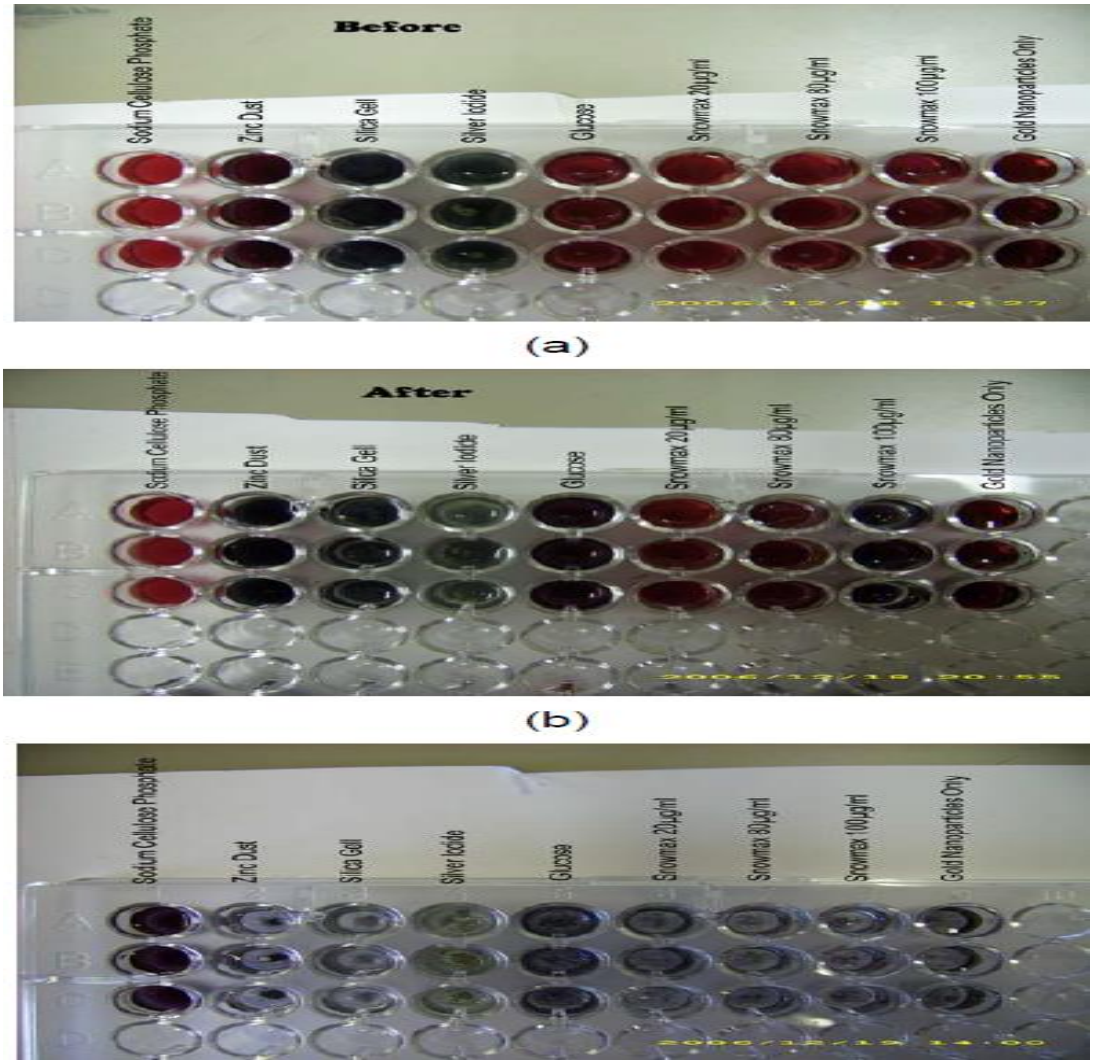


Figure 4.1: Gold nanoparticles with the additives at room temperature (a), near the freezing point (b), after completely freezing then defrosted [60]

4.4.6 Experimental method

The concept of the detection of vaccine freezing temperatures' is based on the use of GNRs, located close to vaccines or eventually inside a Container and refrigerators. In freezing case the GNRs solution were frozen and become colorless due to electron relaxation.If the temperature was changed, the GNRs were converted from frozen manner to colloidal manner and the color converted from colorless to original gold nanoparticles color.

In normal case the gold nanoparticles need long period to change color in freezing manner. An attempt to evaluate nanoparticles has been performed by adding different amount s of some additives like glucose and silica gel. It is expected that the additives will give longer storage time or faster respond to temperature change.

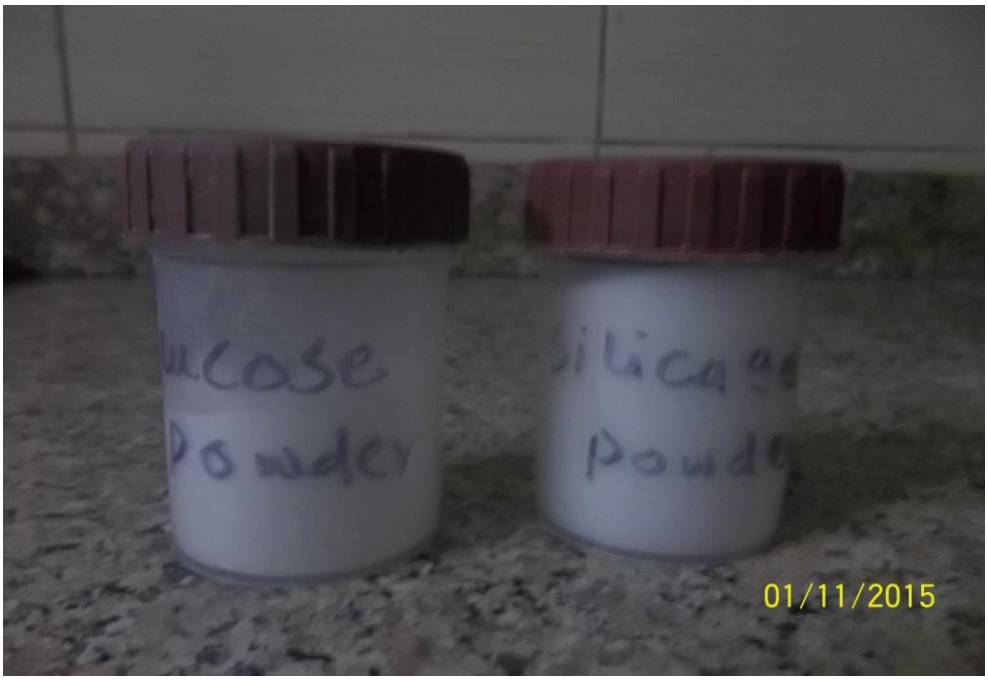


Figure 4.2: powders of glucose and silica gel

4.4.6.1Materials

In this work tow materiales were selected to prepare freezing Glucose [61] and silicagel [62] were provided by Sudan university lab, all chemicals were used . deionized water was used for most of solution preparations

4.4.6.2The instrument

Sensitive balance [KERN Scale] to weighting glucose and silica gel.

Freezer SANYO /ULTRA LOW (-80) and MRI LIBHER (-15) to monitor freezing manners.

4.4.6.3 Experiment detailes

The experiment was prepared by two ways

4.4.6.3.1No additive materials to GNPs solution

Weighted 1 ml of fenugreek GNPs solution and then located in freezer adjusted -15 and then monitor the samples.

4.4.6.3.2Use additive to GNPs solution

Use additive to GNPs solution with size 100mg

Table4.2: additive to GNPs solution with size 100mg

Type of additives	Type of GNP extract	Amount of GNPs solution(ml)	Amount of additive (mg)
Glucose	Fenugreek (6ml)	1ml	100 mg
	fenugreek(10ml)	1ml	100 mg
Silica gel	Fenugreek (10ml)	1ml	100 mg
	Black seed(8ml)	1ml	100 mg

After preparation of the four samples they located in freezer adjusted -15 and then monitor the samples

Use additive to GNPs solution with size 300 and 700 mgs

Table 4.3: additive to GNPs solution with size 300 and 700 mgs of chemical materials

Type of additives	Type of GNP extract	Amount of GNPs solution (ml)	Amount of dionized water (ml)	Amount of additive (mg)
Glucose	Fenugreek (10ml)	1ml	1ml	300 mg
		0.5ml	1.5ml	700 mg
	Black seed(10ml)	1ml	1ml	300 mg
		0.5ml	1.5ml	700 mg
Silica gel	Fenugreek (10ml)	1ml	1ml	300 mg
		0.5ml	1.5ml	700 mg
	Black seed(10ml)	0.4ml	1ml	300 mg
		0.5ml	1.5ml	700 mg

After preparation of the eight samples they located in freezer adjusted -15 and then monitor the samples

4.4.6.4 Result and Discussion

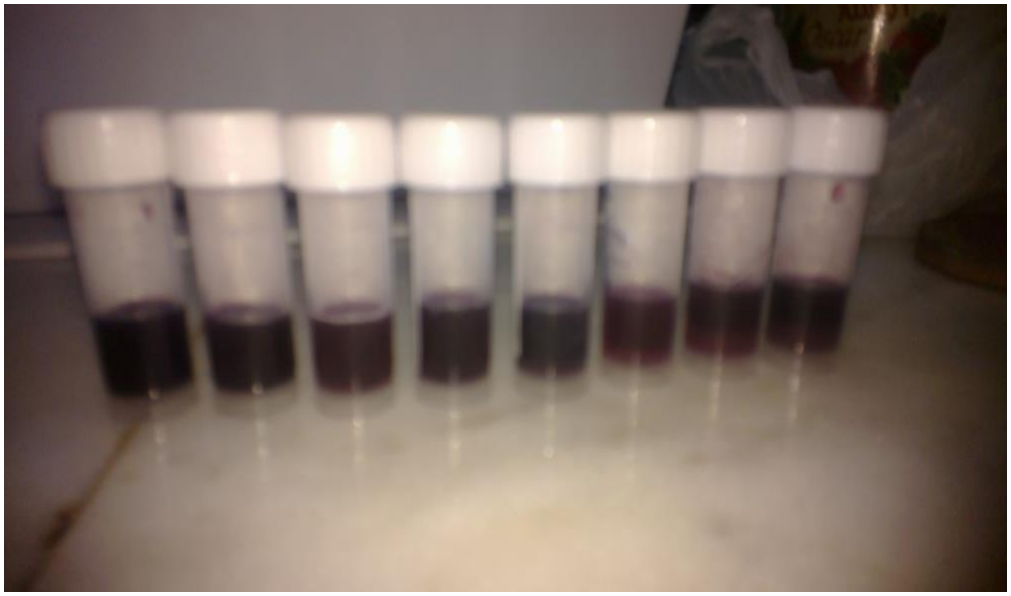
4.4.6 .1 In freezing case:

Found that the samples with adding (100 mg of glucose or silica) in response to the degree of freezing when placed in a refrigerator (-15) the color change during 20 days and slowly. When it was changed to a refrigerator (-80), noted that the sample No. 2 responded to freeze quickly and in just one week and become colorless but note that the GNPs are clustered in the bottom of the tube and the rest of the liquid freezes and change its color. But the rest of the samples observed change did not happen to them.

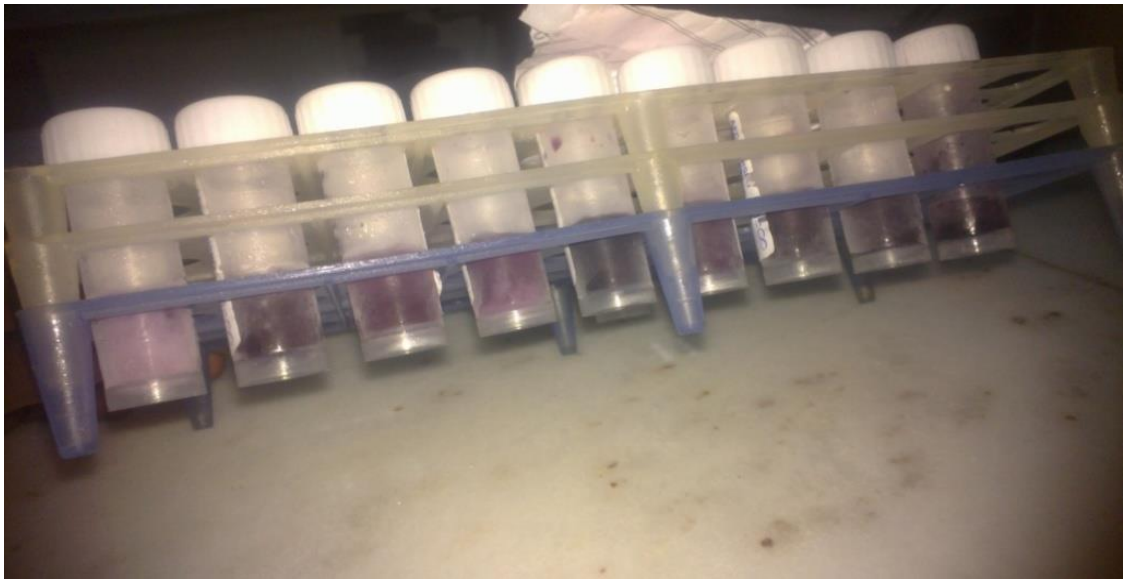
Samples with 300 and 700 mg of glucose and silica gel response to freezing through one week under -15^0 C and -80^0 C.

Samples with added glucose and silica (300mg), No change occur in color except for the sample (300mg of glucose with 0.5ml of GNPs solution).

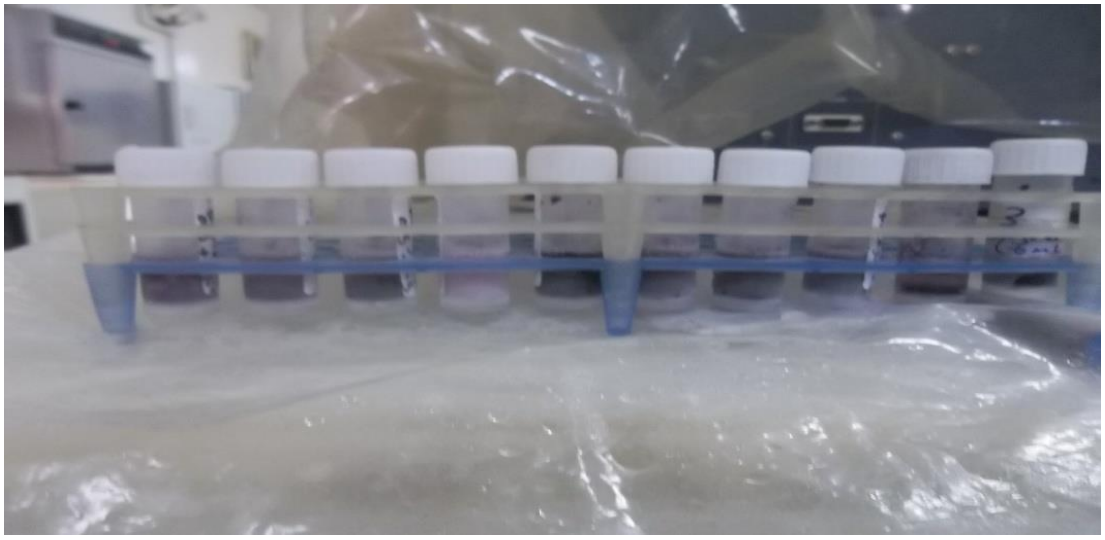
Sample of fenugreek with added(glucose 700mg), found that the samples changed direction to become a colorless, we note that the sample to change color from dark purple to lighter and tended to become colorless response to the freeze and faster when compared to black seed with added (glucose 700mg) (see appendix).



(a)



(b)



(c)

Figure 4.3: (a) Gold nanoparticles with the additives at room temperature (b), near the freezing point within three days (b), after completely freezing after three weeks.

4.4.6.2 In unfreezing case:

Note when outputting samples from the refrigerator at normal temperature, they affected by temperature change within half an hour, found that they came out of the case of freezing to unfreezing, and returned to the original color, found that the solution became in cluster shape grouped down of solution tube .

Finally, found that the more increase the amount of glucose added to the GNPs solution whenever given the change in the color characteristics and faster. As well as the selection of

glucose Itself gives a change in the characteristics and best results when compared to choose silica gel.

also found that the fenugreek in response to the change in temperature faster than black seed , as well as change color characteristics in a short time, which gives a good indication to an application in vaccines to check the degree of preservation and freezing.

4.4.6.5 Conclusion

The development of new synthesis methods for vaccine irreversible detector using cheap and nontoxic chemicals, environmentally benign solvents and renewable materials remains a challenge to the scientific community.

Gold nanoparticles can be proposed as a new alternative for freezing indicator. The suggestion is to stick the freezing indicator on the each packaging of the vaccine. Once the freezing indicator change colour to colourless, that indicates that the vaccine has been exposed to freezing state [60].

In normal case the gold nanoparticles need long period to change color in freezing manner so the suggestion to add some chemical additives to give longer storage time or faster respond to temperature change.

Freezing of nanoparticles is a very complex process that requires a major investigation of the formulation and the process conditions. Many parameters of the formulation may decide the success of freezing as the nanoparticles composition (type of polymer, type and concentration of chemical materials, interaction between chemical materials and nanoparticles solution).

Furthermore, the applied conditions of freezing can impact the stabilization of nanoparticles during and after freezing, especially the temperature, and the duration of each stage of the process.

CHAPTER five

Conclusion and Recommendation

5. Conclusion and Recommendation

5.1 Conclusion

Gold nanoparticles Nanoparticles have wide applications in the field of biomedicine such as drug delivery, imaging, diagnosis and therapeutics due to their extremely small size, high surface area, stability, non-cytotoxicity, physical and chemical properties.

Recent research has been focused on green synthesis methods to produce new nanomaterial, eco-friendly and safer with sustainable commercial viability.

Gold nanoparticle was synthesis using biological method, the black seed, and fenugreek seed extracts as reducing agent for aqueous solution of gold salt and gum Arabic as stabilizer.

The synthesized GNPs are characterized using UV-VIS spectrophotometer, FTIR, transition electron microscope and XRD analysis

Fourier transform infrared spectroscopy (FTIR) measurements were carried out to identify the possible biomolecules in the aqueous extract of seeds, which are responsible for the reduction of the Au+ ions and capping of the resulting Au NPs.

It was found that the concentration of the precursors affects the size of the nanoparticles. The result finding the Typical TEM images obtained for 10 ml black seed colloids consist of almost uniformly sized spherical nanoparticles, while fenugreek consist of different shapes of GNPS.

The XRD studies reflect an interesting feature indicates that gold nanocrystals are highly anisotropic in nature, mainly triangular and hexagonal shapes, and that the particles are (111) oriented.

The development of new synthesis methods for vaccine irreversible detector using cheap and nontoxic chemicals, environmentally benign solvents and renewable materials remains a challenge to the scientific community. Most of the current methods involve known protocols which may be potentially harmful to either environment or human health.

The finding of the present study, the selection of chemical material is important to give good result. also the fenugreek in response to the change in temperature faster than black seed , as

well as change color characteristics in a short time, which gives a good indication to an application in vaccines to check the degree of preservation and freezing.

5.2 Recommendation

This review briefly dealt with the roles of GNPs as detector in vaccines stores. Particular attention, moreover, was given to the temperature as the significant for detection of freezing.

1. Add different amount of glucose and silica gel started from 700mg to give best result of colourless.

2. Analyse the addition of glucose and silica gel to GNP samples by UV-VIS spectroscopy to study optical properties.

3. Synthesis Vaccines container efficiency detector by addition other chemical materials and evaluate it to choose the best one.

4. Designing simple and inexpensive analytical systems to arrive to final shape of detector.

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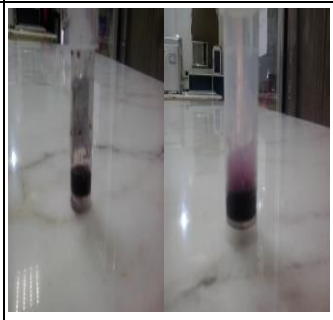
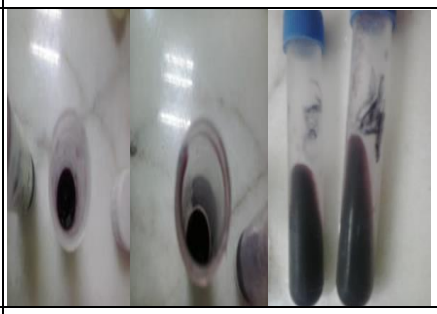
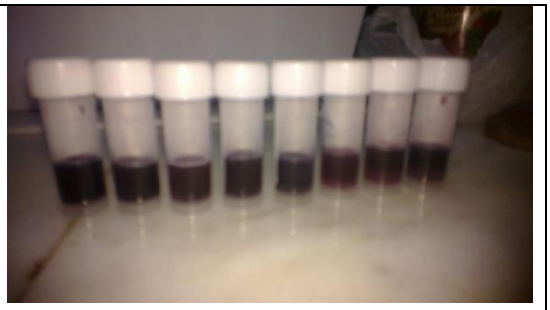
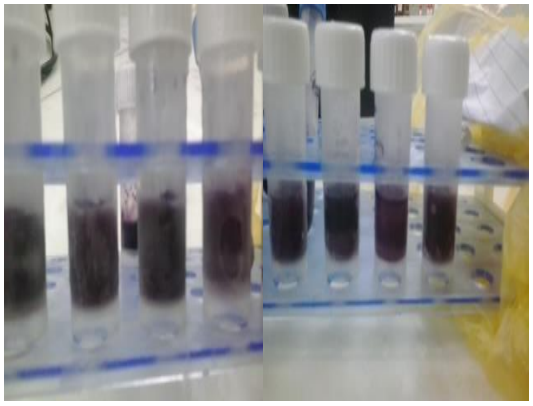
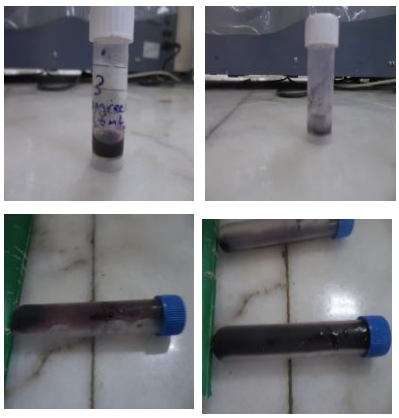
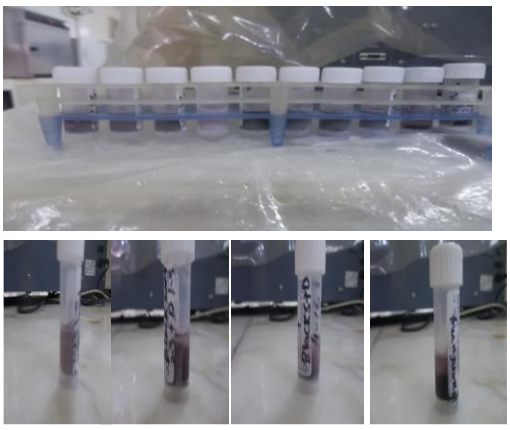
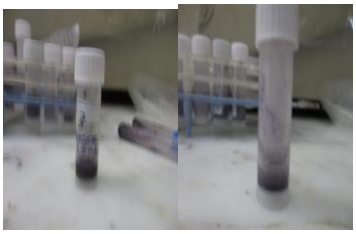
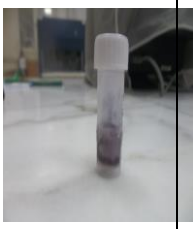
Appendix

date	Images of glucose and silica gel with size 100mg	Images of glucose and silica gel with size 300 and 700 mgs
Day 1		
Day 3		
Day 5		
Day 7	No change	

		
Day 9	No change	
Day 11	No change	
Day 13	The samples unfreezing and they returned to original colors for unexpected malfunction of freezer at (-15^0 C)and then changed to freezer (-80^0 C)	
Day 15		

Day 17		
Day 19		
Day 21		

Day 23	 	   
16.9.2015