Chapter one

Introduction to nanotechnology

1.1 Overview

The term “nano” is derived from the Greek word “nanos” which means small.Nanoscience is the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at a larger scale.’ Nanotechnologies are the design, characterisation, production and application of structures, devices and systems by controlling shape and size at the nanometre scale [1].

According to National Nanotechnology Initiative of the USA, nanotechnology is defined as: research and technology development at the atomic, molecular, or macromolecular levels using a length scale of approximately one to one hundred nm in any dimension; the creation and use of structures, devices and systems that have novel properties and functions because of their small size; and the ability to control or manipulate matter on an atomic scale.

The development of nanotechnologies has been enabled by the invention of two analytic tools that have revolutionized the imaging and manipulation of surfaces at the nanoscale. These are the Scanning Tunneling Microscope (STM) and the Atomic Force Microscope (AFM). The STM and the AFM are capable of imaging surfaces with atomic resolution [1].

1.2 Review for development of nanotechnology

Nanotechnology has its backgrounds in the distant past when people used it without knowledge of it. The word “nanotechnology” was introduced for the first time by Norio Taniguchi at the International Conference on Industrial Production in Tokyo in 1974 in order to describe the super thin processing of materials with nanometer accuracy and the creation of nano-sized mechanisms. Ideas of nanotechnological strategy, which were put forward by Richard Feynman (known as “Father of Nanotechnology”) in his lecture delivered in 1959 at the session of the American Physical Society, were developed by Eric Drexler in 1986. Nanotechnology and nanoscience got a boost in the early 1980s with two major developments: the birth of cluster science and the invention of the Scanning Tunneling Microscope (STM) in

1981. These developments led to the discovery of Fullerenes in 1985 and the structural assignment of Carbon Nanotubes in 1991.In the second half of 1980s and early 1990s a number of important discoveries were made, this created an essential impact on the further development of nanotechnology. In 1991, the first nanotechnological program of National Scientific Fund started to operate in USA. In 2001, the National Nanotechnological Initiative (NNI) of the USA was approved. Since then, lots of scientific and technical research developments have been taking place all over the world especially in countries like Japan, Germany, England, France, China, and South Korea [1].

1.3 The nanometer scale

The nanometer scale is defined as 1 to 100 nm. One nanometre is one billionth of a metre (10-9 m) or mean ‘1 000 times smaller than micro. The size range is normally set to a minimum of 1 nm to avoid single atoms or very small groups of atoms being designated as nano-objects Therefore, nanoscience and nanotechnologies deal with clusters of atoms of 1 nm in at least one dimension.

There is various reasons why nanoscience and nanotechnologies are so promising in materials, engineering and related sciences. First, at the nanometre scale, the properties of matter, such as energy, change. This is a direct consequence of the small size of nanomaterials, physically explained as quantum effects. The consequence is that a material when in a nano-sized form can assume properties which are very different from those when the same material is in a bulk form. Properties like electrical conductivity, color, strength and weight change when the nanoscale level is reached: the same metal can become a semiconductor or an insulator at the nanoscale level. The second exceptional property of nanomaterials is that they can be fabricated atom by atom. Finally, nanomaterials have an increased surface-to-volume ratio compared to bulk materials. This has important consequences for all those processes that occur at the surface of a material, such as catalysis and detection [2].

1.4 Nanoparticle

Nanoparticles are those particles which have two or more than two dimensions and are in the size range of 1 – 100 nm [2]

These particles have special and enhanced physical and chemical properties as compared to their bulk materials due to their large reactive and exposed surface area and quantum size effect as a result of specific electronic structures. These particles have been widely used in many fields such: electronics, medicine, Food industry, Environmental applications and Cosmetics

Once the particle size is reduced below 100 nm they exhibit:

Size effects - Depending on the material used to produce nanoparticles, properties like solubility, transparency, color, absorption or emission wavelength, conductivity, melting point and catalytic behavior are changed only by varying the particle size.

Surface effects - Properties like conductivity, catalytic behavior and optical properties alter with different surface properties of the particle. If the surface properties are not controlled, nanoparticles quickly turn into larger particles due to agglomeration. Most of the size dependent effects are then lost. For the application of nanoparticles, it is therefore crucial to control their agglomeration behavior. Dispersed nanoparticles are needed in order to retain their specific properties for the technological applications.

1.4.1 Properties of nanoparticles

The nanoparticles show various unique features like optical, structural, thermal, mechanical and electromagnetic properties.

Optical properties

As the size of particles size is reduced to nanometer level, depending upon the kind of metal nanoparticles and particle size, they absorb the light with a specific wavelength and due to Surface Plasmon Resonance i.e., the interaction of electromagnetic radiation and the electrons in the conduction band around the nanoparticles they transmit different colors. It is possible to perform quantitative and qualitative analyses of particle size and distributions and particle concentration and the effect of particle shape as consequence of optical spectrum shifts.

The gold nanoparticles show the color phenomena with splendid tinting strength, color saturation and transparency. For instance, bulk gold appears yellow in color, but nanosized gold appears red in color. Furthermore, since the nanoparticles are smaller than the wavelength of visible light and the light scattering by the particles becomes negligible, higher transparency can be obtained with the nanoparticles than the

conventional pigment. As seen in the Raman Effect phenomena, decreasing size of the particle will increase the Kubo gap and therefore the energy emitted by the photons will change frequency and hence their color

Structural properties

The large specific surface area of the nanoparticles is an important property related to reactivity, solubility, sintering performance etc. and is also related with the mass and heat transfer between the particles and their surroundings. Furthermore, the crystal structure of the particles may change with the particle size in the nanosized range in many cases. This is attributed to the compressive force exerted on the particles as a result of the surface tension of the particle itself. The critical particle size of the crystal structure and the size effect differ with the materials.

Thermal properties

As the atoms and molecules located at the particle surface become significant in the nanometer order, the melting point of the material decreases from that of the bulk material because they tend to move easier at the lower temperature. The reduction of the melting point of ultrafine particles is regarded as one of the unique features of the nanoparticles related with aggregation and grain growth of the nanoparticles or improvement of sintering performance of ceramic materials. Hence, melting point of nanomaterials differs from their corresponding bulk materials as an end result of their free surface and size.

Mechanical properties

The hardness of crystalline materials increases with the decreasing crystalline size, and that the mechanical strength of the materials considerably increases by micronizing the structure of the metal and ceramic material or composing them in the nano range.

Electromagnetic properties

When the materials are reduced to the nanolevel, electromagnetic forces become predominant in these nanoparticles. The mass of the nanoscale object is so small, that the gravity becomes negligible and electromagnetic forces overtake the gravitational force. The nanoparticles are raw materials for a number of electronic devices. As for the magnetic property, ferromagnetic fine particles have a single magnetic domain structure as they become very small as in the order less than about 1 μm and show super-paramagnetic property, when they get further finer. In this case

the individual particles are ferromagnetic with the single magnetic domain structure; the particles collectively behave as paramagnetic. It is magnetized as a whole in the same direction of the external magnetic field but the magnetization disappears by the thermal fluctuation, when the external magnetic field is taken away. The time for disappearing of magnetization depends upon the particle size, like, the magnetization of the material responds with the external magnetic field as a paramagnetic when the particles are small enough but it decreases gradually as the particle size becomes larger. Gold which is a stable substance as bulk shows unique catalytic characteristics as nanoparticles.

1.4.2 Gold nanoparticles

The use of gold for medicinal purposes dates back to 2500 BC to the ancient Chinese and Egyptians In medieval Europe, numerous recipes for gold elixirs existed and in the 17^{th} and 19^{th} century gold was used to treat fevers and syphilis, respectively. The use of gold in modern medicine began in 1890 when the German bacteriologist Robert Koch discovered that gold cyanide was bacteriostatic to the tubercle bacillus in vitro. This subsequently led to the treatment of tuberculosis with gold in the early 20^{th} century. Properties of gold nanoparticles are different from its bulk form because bulk gold is yellow solid and it is inert in nature while gold nanoparticles are wine red solution and are reported to be anti-oxidant. Inter particle interactions and assembly of gold nanoparticles networks play key role in the determination of properties of these nanoparticles [3].

Gold nanoparticles exhibit various sizes ranging from 1 nm to 8 μm and they also exhibit different shapes such as spherical, sub-octahedral, octahedral, decahedral, icosahedral multiple twined, multiple twined, irregular shape, tetrahedral, nanotriangles, nanoprisms, hexagonal platelets and nanorods Among all these shapes triangular shaped nanoparticles show attractive optical properties as compared to the spherical shaped nanoparticles.

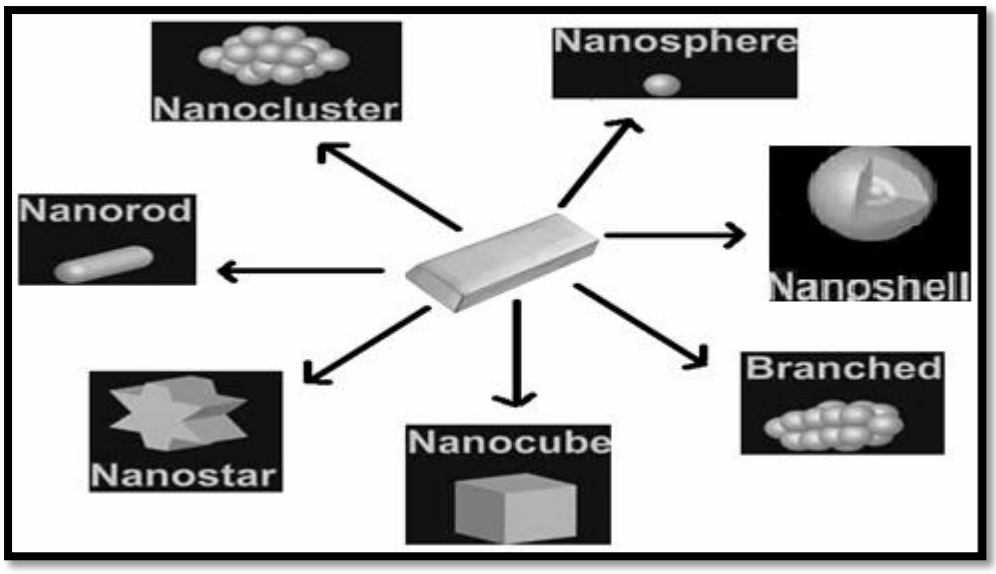

Figure 1.1: Shapes of gold nanoparticles.[21]

1.4.3 Optical & Electronics Properties of GNPs

Gold nanoparticles interaction with light is strongly dictated by their environment, size and physical dimensions. Oscillating electric fields of a light ray propagating near a colloidal nanoparticle interact with the free electrons causing a concerted oscillation of electron charge that is in resonance with the frequency of visible light. These resonant oscillations are known as surface plasmons. For small (~30nm) monodisperse gold nanoparticles the surface plasmon resonance phenomona causes an absorption of light in the blue-green portion of the spectrum (~450 nm) while red light (~700 nm) is reflected, yielding a rich red color. As particle size increases, the wavelength of surface plasmon resonance related absorption shifts to longer, redder wavelengths. Red light is then absorbed, and blue light is reflected, yielding solutions with a pale blue or purple color .As particle size continues to increase toward the bulk limit, surface plasmon resonance wavelengths move into the IR portion of the spectrum and most visible wavelengths are reflected, giving the nanoparticles clear or translucent color. The surface plasmon resonance can be tuned by varying the size or shape of the nanoparticles, leading to particles with tailored optical properties for different applications.

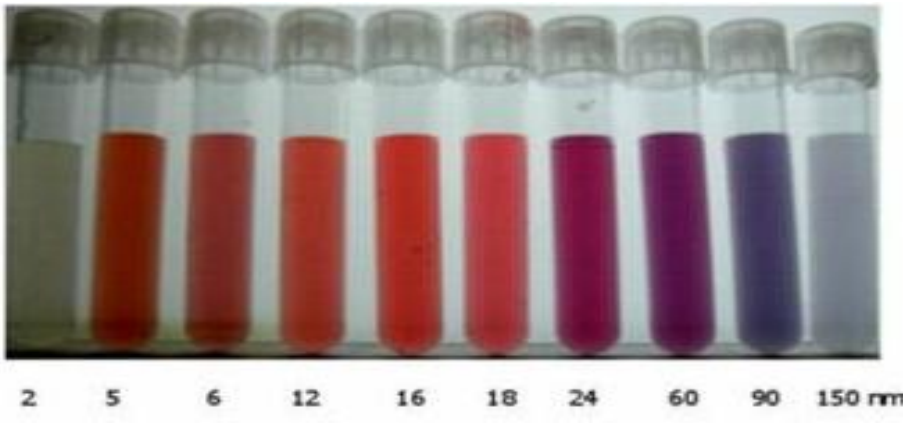

Figure 1.2: Colors of various GNPs sized.[31]

1.4.4 Applications of GNP s

The field of nanotechnology mainly encompasses with biology, physics, chemistry and material sciences and it develops novel therapeutic nanosized materials for biomedical and pharmaceutical applications [4] the range of applications for gold nanoparticles is growing rapidly. Gold nanoparticles are widely used in biomedical science according to its physiochemical properties biocompatibilities and surface carrier capabilities which make them suitable for use in medical applications.the application of GNPs are

1. Electronics – nanoparticles are important components in the chip design.
2. Photodynamic Therapy. When light is applied to a tumor containing gold nanoparticles, the particles rapidly heat up, killing tumor cells in a treatment also known as hyperthermia therapy.
3. Therapeutic Agent Delivery - agent can be coated onto the surface of gold nanoparticles.
4. Sensors Gold nanoparticles are used in a variety of sensors.
5. Probes - Gold nanoparticles also scatter light and can produce an array of interesting colors under dark-field microscopy.
6. Diagnostics - Gold nanoparticles are also used to detect biomarkers and common in lateral flow immunoassays.
7. Catalysis - Gold nanoparticles are used as catalysts in a number of chemical reactions.
9. Magnetic Separation and Detection [5].

1.5 Problem Statement.

Nanotechnology has played a crucial role in the development of biosensors over the past decade. The development, testing, optimization, and validation of new biosensors have become a highly interdisciplinary effort involving experts in chemistry, biology, physics, engineering, and medicine and need large number of trials to overcome the best result .

1.6 Objectives

The main objective of the current study is to develop a mathematical model for immunochromatograply assay. The details are described below

1-Synthesis nontoxic and low cost gold nanoparticles using plant seed extraction

2- Characterize GNPs by TEM images, and different types of spectroscopes (UV-VIS, XRD, EDX, and FTIR)

3-Produce biosensor model.

4-Study the performance of the sensor in different concentrations and times.

1.7 Method of research

Figure (1, 4) summarizes the experimental part of the research work

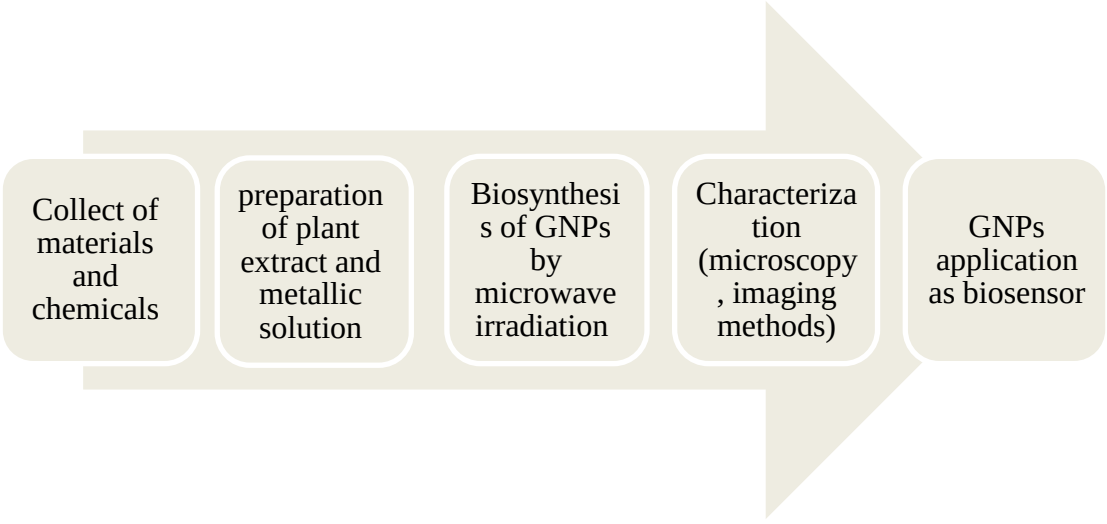

Figure 1.3: The steps of the research

1.8 Layout

This thesis contains five chapters writing as papers . Chapter one is general introduction to nanotechnology and general view of the project .Chapter two about the synthesis methods of the nanoparticle and the used method in this project .Chapter three presents the characterization methods used in this project .Chapter four the application of nanoparticles in biosensor and mathematical model of the sensor. Finally, chapter five highlights the summary and future work.

Chapter two

Synthesis of Nanoparticles

2.1 Introduction

Nanoparticles are broadly classified in to two categories,

- 1-Organic nanoparticles include carbon nanoparticles
- Inorganic nanoparticles include metal nanoparticles (Ag, Au, Pt, and Pd), magnetic nanoparticles and semi-conductor nanoparticles (TiO2, SiO2, and ZnO2).

There are two distinct approaches to making products with nanoscale features

Top-down fabrication is the method used in the microelectronics industry, where small features are created on large substrates by repeated pattern transfer steps involving lithographic methods. Extreme UV photolithography can produce patterns with feature sizes down to 100 nm, and electron beam lithography can be used for features down to 30 nm.

Bottom-up fabrication is directly relevant to the chemicals industry. This method starts with very small units, often individual molecules or even atoms, and assembles these building-block units into larger structures clearly the domain of chemistry

In both methods, two requisites are fundamental: control of the fabrication conditions (e.g. energy of the electron beam) and control of the environment conditions (presence of dust, contaminants, etc.). For these reasons, nanotechnologies use highly sophisticated fabrication tools that are mostly operated in a vacuum in clean-room laboratories.

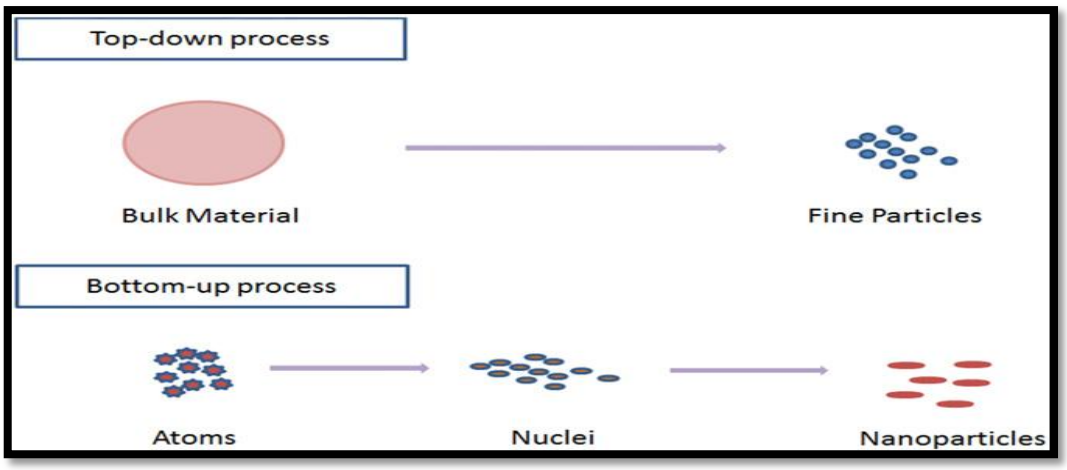

Figure 2.1: Methods of fabrication nanoparticles.[32]

2.2 Methods of synthesis of gold nanoparticle

Gold Nanoparticles traditionally synthesized by reducing metallic gold in +3 residues state to nanoparticulate gold .The synthesis is done by

1-Chemical methods

Means use chemicals in synthesis of NPs, Most important synthesis agents are used Tri sodium citrate (Citrate synthesis) and sodium borate (Borate synthesis).

2-Physical methods

Done by breaking down the bulk material to nanoscale, methods are including plasma arcing, ball milling, thermal evaporate, spray pyrolysis, ultra thin films, pulsed laser desorption, lithographic techniques, sputter deposition, layer by layer growth, molecular beam epistaxis and diffusion flame synthesis of nanoparticles [4].

3-Green methods (biological syntheses)

The biological syntheses of nanoparticles are being carried out by different macromicroscopic organisms such as plant, bacteria, fungi, seaweeds and microalgae. Most of the non-biological methods (chemical and physical) are used in the synthesis of nanoparticles, which has a serious hazardous and high toxicity for living organisms. In addition, the biological synthesis of metallic nanoparticles is inexpensive, single step and eco-friendly methods**.** The plant contains abundant natural compounds such as alkaloids, flavonoids, saponins, steroids, tannins and other nutritional compounds. These natural products are derived from various parts of plant

such as leaves, stems, roots shoots, flowers, barks, and seeds. Recently, many studies shave proved that the plant extracts act as a potential precursor for the synthesis of nanomaterial in non-hazardous ways. Since the plant extract contains various secondary metabolites, it acts as reducing and stabilizing agents for the bioreduction reaction to synthesized novel metallic nanoparticles. [4].Biosynthesis reaction can be altered by wide range of metal concentration and amount of plant extract in the reaction medium, it may transform the shapes and size of the nanoparticles.

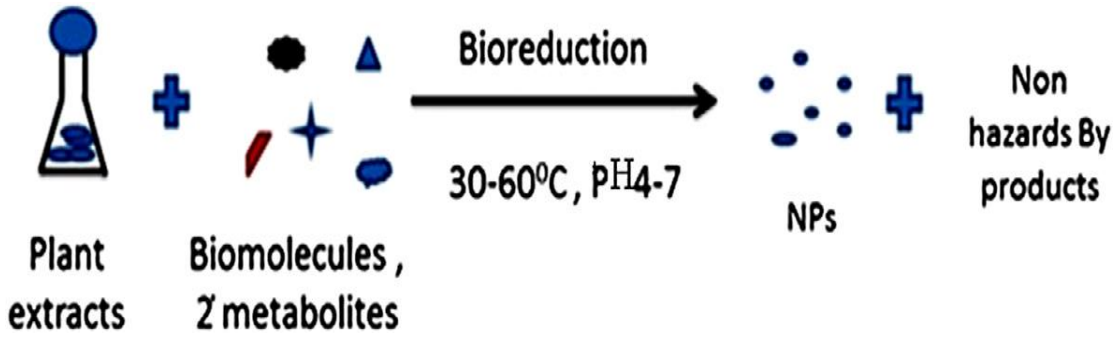

Figure 2.2: Mechanism of synthesis nanoparticles using plant extracts**.**[4]

2.3 fenugreek seeds (*Trigonella foenum*)

It is a winter season crop and grows well in low temperature. Fenugreek seeds are traditionally used for the treatment of many diseases. Studies show that the seeds have antioxidant properties. Many medicinal properties are attributed to fenugreek seeds and leaves . Fenugreek is known to have several pharmacological attributes such as hypoglycaemic, hypercholesterolaemic , gastroprotective , chemo-preventive , antioxidant , laxative , and appetite stimulation ,The plant is known to contain alkaloids , flavonoids , salicylate , and nicotinic acid **.**

The fenugreek seed extract contains high flavonoids and other natural bioactive products such as lignin, saponin and vitamins. The reduction of chloroauric acid by using the powerful reducing agents fenugreek seed extract acts as a better surfactant.

The COO_ group (carboxylic), C,N and C,C functional groups are present in the seed extract. The functional group of metabolites acts as a surfactant of gold nanoparticles and the flavonoids can stabilize the electrostatic stabilization of gold NPs[4].

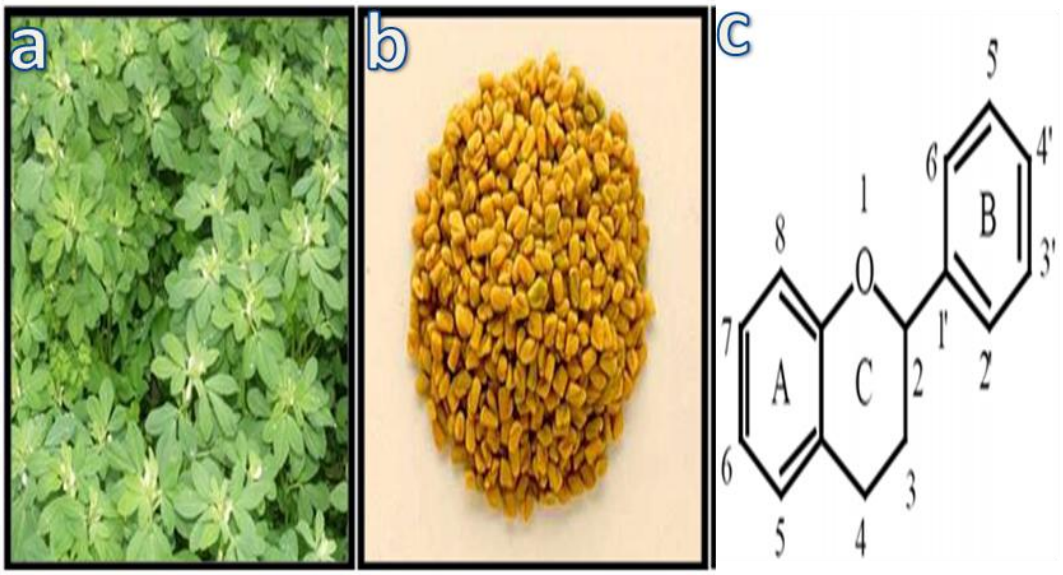

Figure 2.3: Fenugreek (*Trigonella foenum-graecum*) plant a, dried seeds b and flavonoids structure c.[11]

2.4 Black seed (*Nigella sativa*)

Black cumin (*Nigella sativa* L., Family: Ranunculaceae) is an annual herb possessing wide range of medicinal uses apart from its commercial significance as a spice yielding plant. Black cumin seeds are used in folk (herbal) medicine all over the world for the treatment and prevention of a number of diseases. Prophet Mohammad (Peace be Upon Him) said: "Use this Black Seed; it has a cure for every disease except death" (Sahih Bukhari). The plant species is also important cytogenetically and may be used as a model plant for better understanding of gene and chromosome relationship. Despite the major advancement of modern medicine in human health-care, it is still intangible and beyond reach to ailing humanity, especially the destitute and therefore in recent years plant based system has been utilized for traditional medicine and phytotherapy. 'Medicinal plants are gift of nature' and black cumin is one such plant with potential uses, which can be explore for safe and effective herbal medicine for human benefit. Considering nearly all essential aspects of the species (synonym(s), common names, origin of the name, distribution, varieties, plant description, floral biology, pollination biology, scanning electron microscopy of seed surfaces, cultivation, economy, diseases, pest, microscopical and powdered characteristics, biochemical constituents, extraction methods of essential oils,

therapeutic uses, insecticidal activity, other uses, clinical trials, biosafety, tissue culture and patents), a monograph is prepared on the laid formulation of WHO (World Health Organization) as well as on other significant parameters (cytogenetics and molecular genetics) with the following objectives: to provide an unabridged repository of references regarding the species for its effective and safe utilization as a 'Potential Medicinal Herb'; for creating awareness regarding the use of plant based medicine; understanding economic status, biosafety and patents for regulating herbal medicinal market Nationally and Internationally and exploration of cytogenetical and genetical aspects.

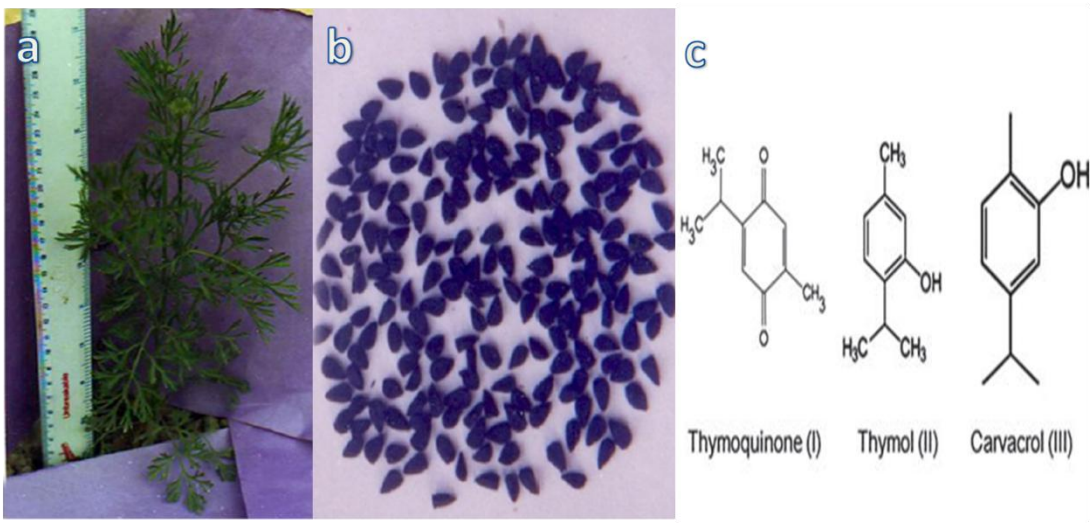

Figure 2.4: Nigella sativa plant a, black seeds b, and reduction characteristics c [9, 12]

2.5 Microwave-Assisted synthesis of GNPs

Microwaves are the electromagnetic waves with frequencies ranging from 0.3 to 300 GHz and with wavelengths of between 1 mm and 1 m, which are between infrared and radio frequency waves in the electromagnetic spectrum. The commonly used frequency in laboratories and homes for microwave heating is 2.45 GHz (with a wavelength of about 12.24 cm.

.The microwave heating technology is emerging as an alternative popular heat source for rapid chemical reactions and materials synthesis in minutes, instead of hours or even days usually required by the conventional heating methods [7].

2.6 Review of synthesis of GNPs by Fenugreek and black seed extract

2.6.1 Black seeds

In 2011 Sneha et al describes for the first time use of cumin seed powder for synthesis of gold nanoparticle and nanoplatelets. They also scrutinized the effect of temperature and pH, followed by investigation on the chemical state of the nanoparticle formed. The effects of pH and temperature on biosynthesis were performed at various initial pH ranging from 1–9 and temperature ranging from 0 to 100 ºC.

In 2012 Fragoon, et al developed a rapid and non-toxic method for the preparation of biocompatible gold nanoparticles using Black Seed (Nigella Sativa) Extract by two different synthetic routes: microwave irradiation and thermo-induced procedures. [9]

2.6.2 Fenugreek seeds

In 2012 S. Aswathy et al development 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. This research focused on green synthesis methods to produce new nanomaterials, ecofriendly and safer with sustainable commercial viability. They report 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. 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 [10].

2.7 The materials and methods

2.7.1 Preparation of plant seed extract and metallic solution

Plant seeds purchased from local herbal shop and golden chloride purchased from lab course company (Sudan). 8grams of the plant seeds were weighted and washed with deionized water to remove any contaminant or dust particles. , placed on filter paper to dry. The beaker washed with deionized water and drying in an oven. The plant seed placed in 50 ml deionized water at beaker and covered with plastic cover. Black seeds were incubated for 72 h and fenugreek incubated for 24 h, at room temperature after the incubation period, the supernatant was decanted and centrifuged at 6000 rpm for 15 min at room temperature. Stored at 4 °C and used within 3 days. The metallic solution prepared by adding 1 g of HAuCl4.3H2O powder to29ml of deionized water in 50ml beaker.

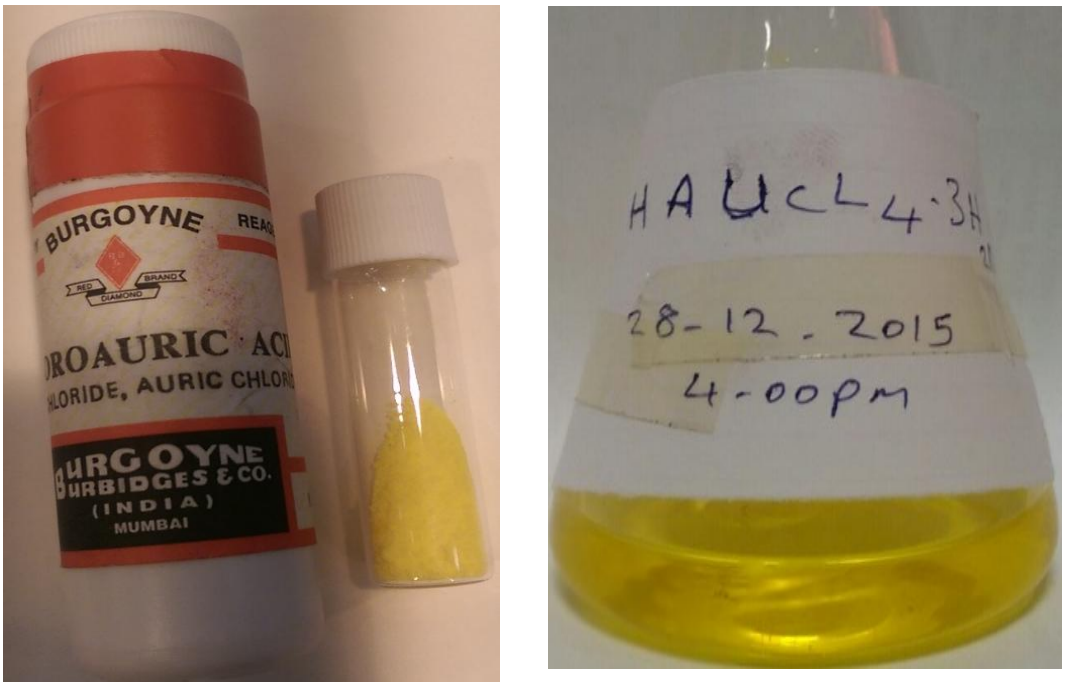

Figure 2.5: Golden chloride and metallic solution

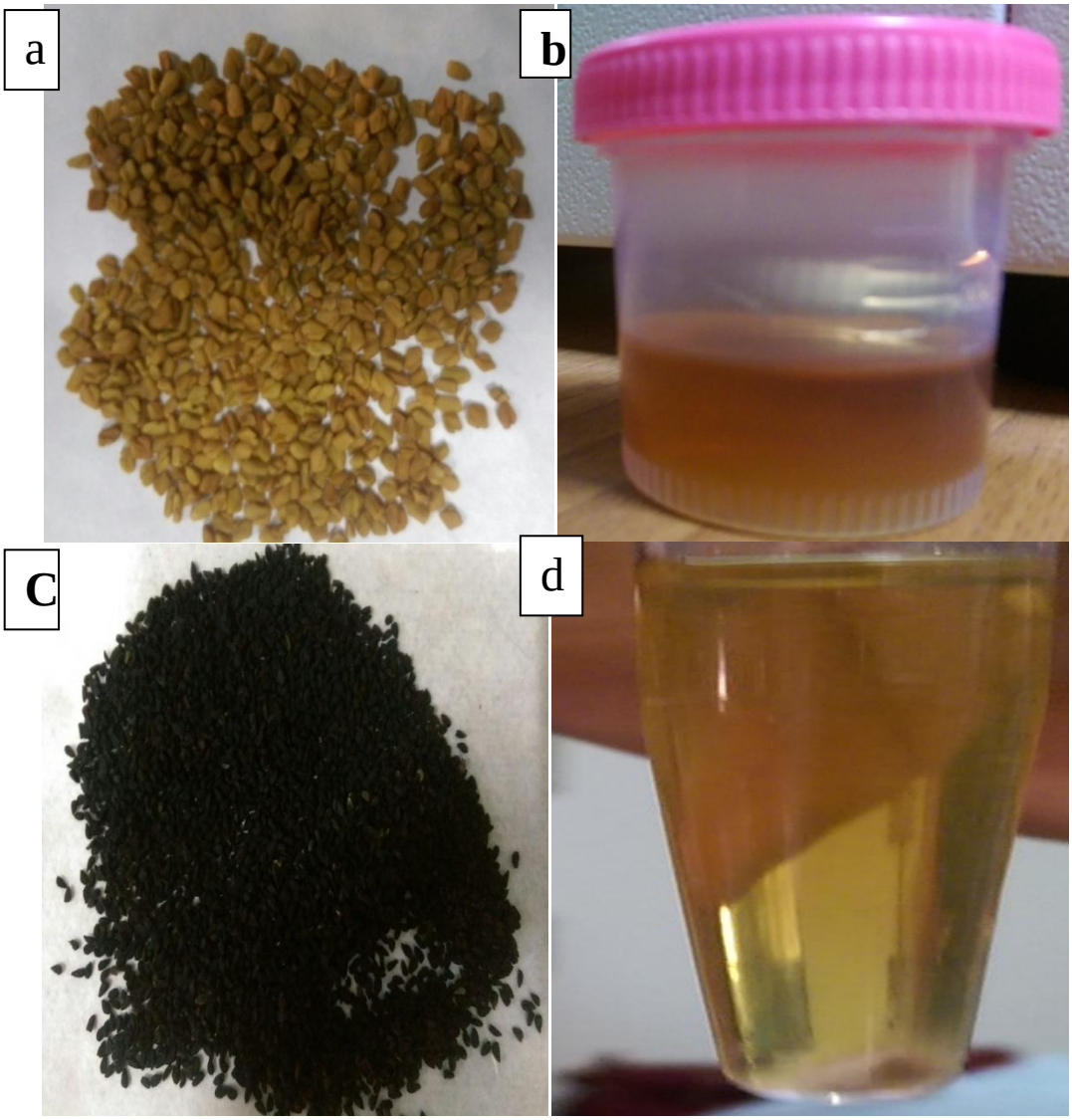

Figure 2.6: fenugreek seed (a), fenugreek seed extract (b), black seed (c), black seed extract (d)

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Mixing of metallic solution with plant extract

To 250 ml beaker adding:

Type of additive	**volume**
Plant seeds extract	10ml or 8ml or 6ml
Deionized water	10ml or 12ml or 14ml
Gum Arabic	120mg
Metallic solution	1.16ml

2.7.2 Biosynthesis of GNPs by Microwave Irradiation

The beaker was placed in the center of microwave oven (LG) at 2450 MHz for 30s for black seeds samples and fenugreek sample. The color of the solution turned from pale yellow to purple-red indicating the formation of GNPs.the solution then left to cool to room temperature, after 2min the reduction was completed indicated by stable light purple –red color of the solution.

2.8 Results

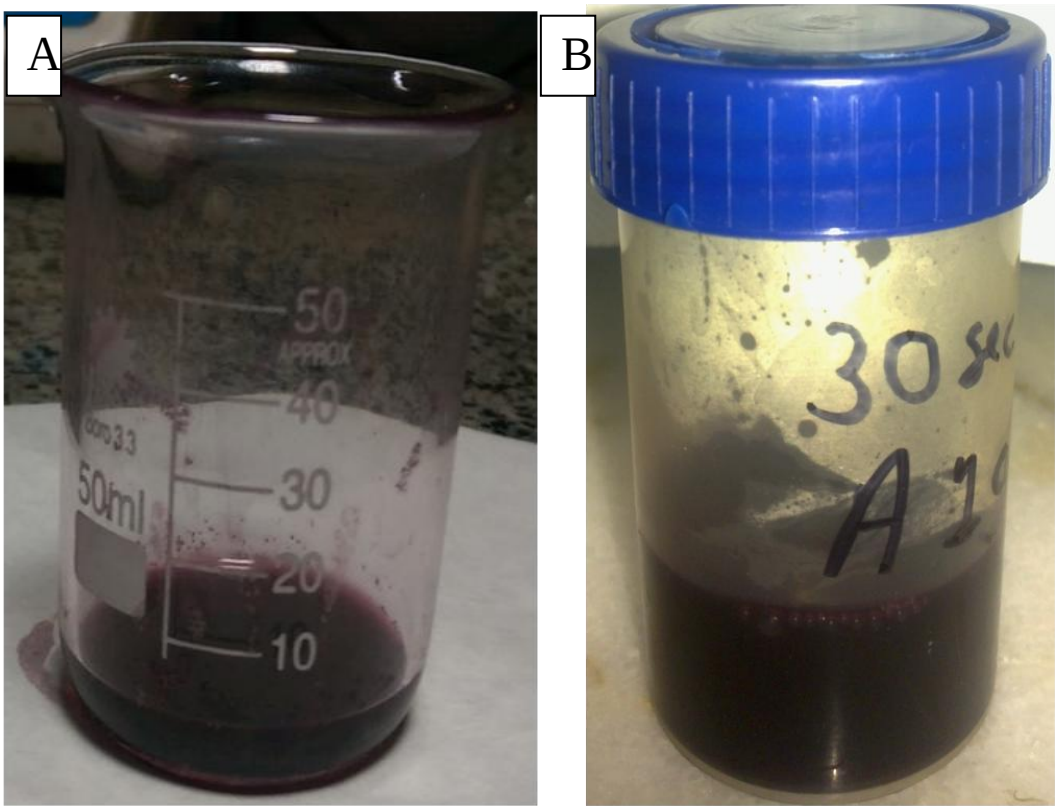

Figure 2.7: Synthesized GNPs by microwave, black seed 10m (a), fenugreek 10ml (b).

Addition of plant extract to 0.1m M aqueous HAuCl4solution, lead to the formation of ruby red color that clearly indicated the presence of GNPs. It is well known that gold nanoparticles exhibit a ruby-red color in aqueous solution due to the excitation of surface plasmon resonance in GNPs.

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2.9 Conclusion

Physical and chemical methods are more popular in the synthesis of nanoparticles,the use of toxic chemicals greatly limits their biomedical applications, in particular in clinical fields. Therefore, development of reliable, nontoxic, and eco-friendly methods for synthesis of nanoparticles is importance to expand their biomedical applications. One of the options to achieve this goal is to use plants to synthesize nanoparticles.

The method of synthesis GNPs mentioned here has advantages such as rapidness, cheap, environmentally benign solvents, renewable materials and, most important, resulting in biocompatible GNPs. Because the synthesized GNPs have low toxicity compared to chemical method.

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Chapter three

GNPs characterization

3.1 Introduction

Imaging of nanomaterials is an essential part of nanoscience and nanotechnologies. Imaging in nanoscience does not just mean ‘to create an image’, but to understand its meaning. Scientists nowadays have access to a variety of truly instruments that allow them to see objects at the nanoscale. This was a dream for scientists until just a couple of decades ago, a dream that came true in the mid 1980s when a revolutionary instrument was invented, the scanning tunnelling microscope, and shortly after, the atomic force microscope. As a matter of fact, it was the invention of these instruments that truly opened the doors to the nano-world. Once scientists were able to see nanoscale objects, they started to be able to analyse them, understand their behavior, and imagine ways of manipulating them [1].

There are many methods available to image nanostructured materials (e.g. a nanostructured surface) and to characterize their physical and chemical properties. In general, two fundamental types of characterization methods exist: imaging by microscopy and analysis by spectroscopy.There are various types of electron microscopes, such as the scanning electron microscope (SEM) and the transmission electron microscope (TEM). Spectroscopy such as X-ray methods, UV-visible plasmon absorption and emission, Plasmon resonance light scattering, Surface-enhanced Raman scattering. [1]

3.2 Characterization instruments

3.2.1 UV/visible spectrometer

Ultraviolet and visible spectrometers have been in general use for the last 35 years and over this period have become the most important analytical instrument in the modern day laboratory. In many applications other techniques could be employed

but none rival UV-Visible spectrometry for its simplicity, versatility, speed, accuracy and cost-effectiveness.

The processes concerned in absorption spectrometry are absorption and transmission. Usually the conditions under which the sample is examined are chosen to keep reflection, scatter and fluorescence to a minimum. In the ultraviolet and visible regions of the electromagnetic spectrum, the bands observed are usually not specific enough to allow a positive identification of an unknown sample, although this data may be used to confirm its nature deduced from its infrared spectrum or by other techniques. Ultraviolet and visible spectrometry is almost entirely used for quantitative analysis; that is, the estimation of the amount of a compound known to be present in the sample. The sample is usually examined in solution.

In general terms there are two major measurement techniques; how much analyte is in the sample (quantitative analysis) and which analyte is in the sample (qualitative analysis).

For quantitative analysis, it is normally chosen to use radiation of a wavelength with extinction coefficient, is a maximum, at the peak of the absorption band, for the reason of the change in absorbance for a given concentration change is greater, leading to greater sensitivity and accuracy in measurement[13].

3.2.2 Energy dispersive X-ray spectroscopy (EDX)

As a type of spectroscopy, it relies on the investigation of a sample through interactions between electromagnetic radiation and matter, analyzing x-rays emitted by the matter in response to being hit with charged particles.

3.2.3 X-ray Diffractometer (XRD)

About 95% of all solid materials can be described as crystalline. When x-rays interact with a crystalline substance (Phase), one gets a diffraction pattern.

Diffraction occurs when light is scattered by a periodic array with long-range order, producing constructive interference at specific angles.

of electrons around the atom.

• The atoms in a crystal are arranged in a periodic array and thus can diffract light. The wavelength of X rays is similar to the distance between atoms. The scattering of

X-rays from atoms produces a diffraction pattern, which contains information about the atomic arrangement within the crystal .Amorphous materials like glass do not have a periodic array with long-range order, so they do not produce a diffraction pattern.

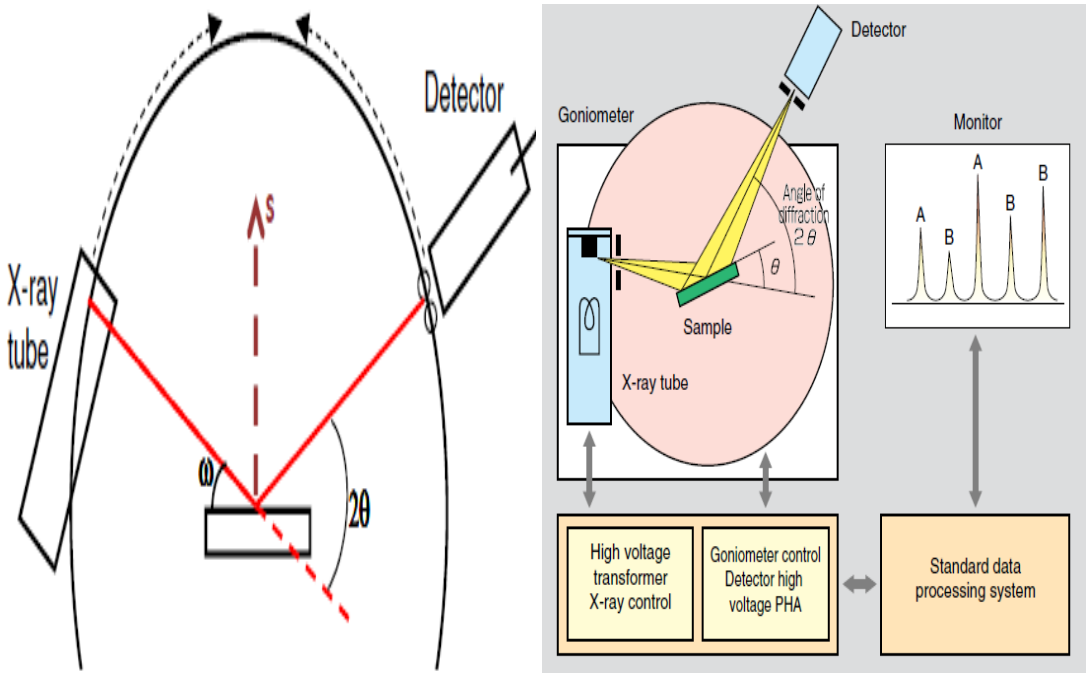

Figure 3.1: XRD basic diffraction parts and instrument parts.[14]

The incident angle, ϕ, is defined between the X-ray source and the sample.

• The diffraction angle, 2Θis defined between the incident beam and the detector.

• The incident angle ϕ is always of the detector angle 2Θ.

– In aΘ: 2Θ instrument the tube is fixed, the sample rotates at Θ°/min and the detector rotates at 2Θ °/min.

– In a Θ: Θ instrument the sample is fixed and the tube rotates at a rate –Θ °/min and the detector rotates at a rate of Θ°/min.

• In the Bragg-Brentano geometry, the diffraction vector (s) is always normal to the surface of the sample.

– The diffraction vector is the vector that bisects the angle between the incident and scattered beam [14].

3.2.4 Fourier transforms infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is a technique which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. FT-IR stands for Fourier Transform Infrared, the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some is transmitted. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. An FTIR spectrometer simultaneously collects spectral data in a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. Fourier transform spectroscopy is a less intuitive way to obtain the same information.

Rather than shining a monochromatic beam of light at the sample, this technique shines a beam containing many different frequencies of light at once, and measures how much of that beam is absorbed by the sample. Next, the beam is modified to contain a different combination of frequencies, giving a second data point. This process is repeated many times. Afterwards, a computer takes all these data and works backwards to infer what the absorption is at each wavelength [15].

This instrument covered the wavelength range from 2.5 µm to 15 µm and the wave number range is between 4000 cm^{-1} c to 660 cm^{-1}. For the relatively long wavelengths of the far infrared is about ~ 10 µm and the near-infrared region spans the wavelength range between at about 750 nm. It is used mainly in industrial applications such as process control and chemical imaging. FTIR can be used in all applications where a dispersive spectrometer was used in the past. It is used to identify unknown materials, determine the quality or consistency of a sample and can determine the amount of components in a mixture. Fourier transform infrared spectroscopy is preferred over other methods of infrared spectral analysis for several reasons:

i. It is a non-destructive technique
ii. It provides a precise measurement method which requires no external calibration
iii. It can increase speed, collecting a scan every second

iv. It is mechanically simple with only one moving part

Because there needs to be a relative scale for the absorption intensity, a background spectrum must also be measured. This is normally a measurement with no sample in the beam. Thus, all spectral features which are present are strictly due to the sample. A single background measurement can be used for many sample measurements because this spectrum is characteristic of the instrument itself. Quantitative methods can be easily developed and calibrated and can be incorporated into simple procedures for routine analysis [15].

3.2.5 Transmission electron microscopy (TEM)

Electron microscopes are scientific instruments that use a beam of energetic electrons to examine objects on a very fine scale. The transmission electron microscope (TEM) was the first type of Electron Microscope to be developed and is patterned exactly on the light transmission microscope except that a focused beam of electrons is used instead of light to "see through" the specimen. It was developed by Max Knoll and Ernst Ruska in Germany in 1931. Transmission electron microscopy is typically used for high resolution imaging of thin films of a solid sample for nanostructural and compositional analysis. 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. 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 semi crystalline material and propagated along different directions

(ii) Imaging and angular distribution analysis of the forward-scattered electrons (unlike SEM where backscattered electrons are detected)

(iii) Energy analysis of the emitted X-rays.

The principal setup of TEM is similar to one of light microscope. The sample is illuminated with the light (with electrons in case of TEM). The image of the sample is then magnified by means of projection lenses and represented on a canvas (screen). However in TEM electromagnetic lenses are used in case of glass lenses to guide the electron beam through the microscope. The principal setup of TEM is illustrated on the figure (3.2) Different parts of the microscope contribute to image formation:

illumination (FEG, Condensor Lenses C1 and C2), image formation due to objective lenses, projection system (projective lenses) and image acquisition (CCD) [16].

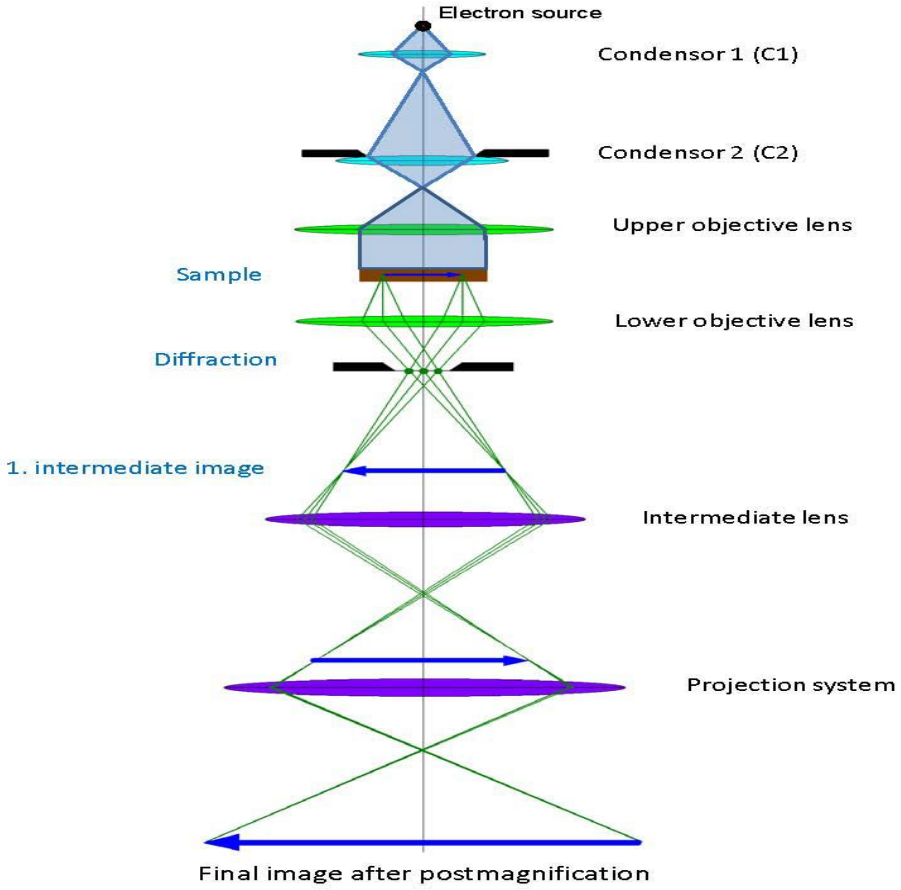

Figure 3.2: Different parts of the microscope[15]

The illumination system situates in the top part of microscope and consists of electron source and condensor lenses (C1 and C2). The illumination system takes the electrons from the source and transfer them to the sample in form of parallel or convergent beam. Usually the following electron sources are used: thermionic sources like LaB6 or tungsten sources and field emission gun (FEG). All of them have some advantages and disadvantages. However nowadays modern microscopes are normally equipped with FEG, because this source produces very bright electron beam by low operating temperature and have very long lifetime.

After electron source we have system of condenser lenses. These lenses are used in order to form the electron beam which illuminates the sample. By changing the focal distance of condenser lenses we can create parallel or convergent illumination. The parallel beam is usually used in traditional TEM. The convergent beam is typical

for STEM mode (Scanning TEM), because one needs to illuminate every point of the sample. Here the most important parameter is semi-convergence angle _. It also plays very important role in image simulations.

Objective lenses of the microscope are used for image formation. After propagating through objective lens all electron beams from the sample are focused at the image plane. Here one uses objective aperture in order to exclude electrons at high scattering angles. In HRTEM mode usually no objective apertures are used. The objective lenses define the quality of imaging and in that way the resolution of microscope.

The real lenses suffer from different aberrations. The most important one is spherical aberration Cs.The projection system of microscope consists of intermediate lens and projection lenses. By changing the strength (i.e. the focal distance) of intermediate lens it's possible to switch between diffraction and imaging modes. If the back focal plane of objective lenses corresponds to the object plane of intermediate lens we can obtain diffraction on the viewing screen. In imaging mode we readjust the intermediate lens so that its object plane is the image plane of objective lens. The projection lenses are used for post-magnification of the image. By HRTEM the highest magnification can be 106 or even higher. The final image is recorded with CCD. [16].

3.3 The method and materials

UV/visible spectroscopy

Optical absorption spectra of the synthesized GNPs (by black seed or fenugreek seed extract) were recorded using the UV-VIS-1800 Spectrophotometer (Shimadzu, Japan) Optical absorption spectra of the with 2 ml of GNPs solution in a 1 cm optical path cuvette.

Figure 3.3: UV/visible-1800 Spectrophotometer (Shimadzu, Japan)

XRD

The Solution was centrifuged at 13,000 rpm for 15 min to obtain concentrated GNPs then was put on a glass slide and air dried at ambient conditions. The XRD pattern of the dried sample was obtained with the help of an XRD instrument (labx, XRD-6000) in thin-film mode with Cu source. The relative intensity was recorded in the scattering range (2θ) of 10–90◦ in steps of 0.1. Operated in voltage 40 kv, and current 30 mA

Figure 3.4: lab x, XRD-6000

TEM images

A few drops of GNPs suspension was placed on a carbon-coated copper grid and air dried at room temperature. TEM, high-resolution transmission electron microscopy (HRTEM) measurements were performed on a TEM instrument (JEOL-JEM-2100Tokyo, Japan) operated at 200 kV.

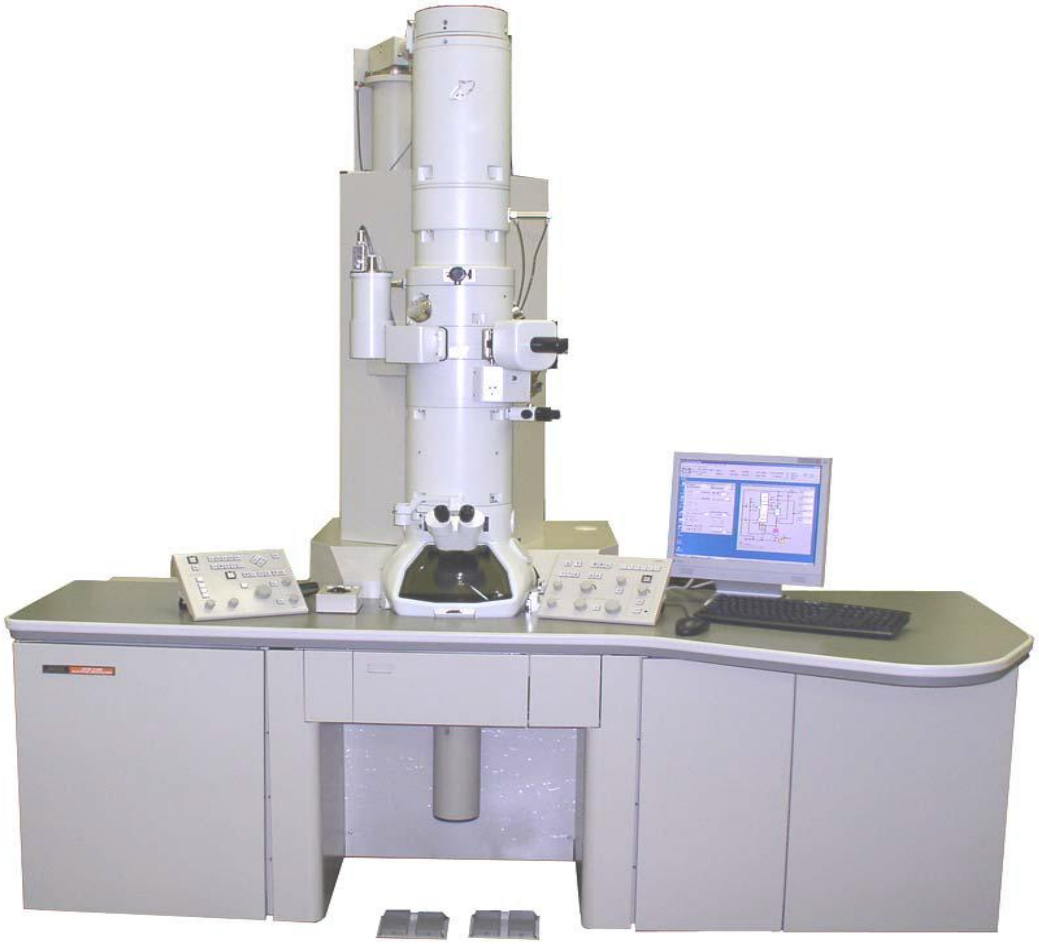

Figure 3.5 :TEM (JEOL-JEM-2100).[35]

3.4 Review of characterization

Black seed

Sneha method

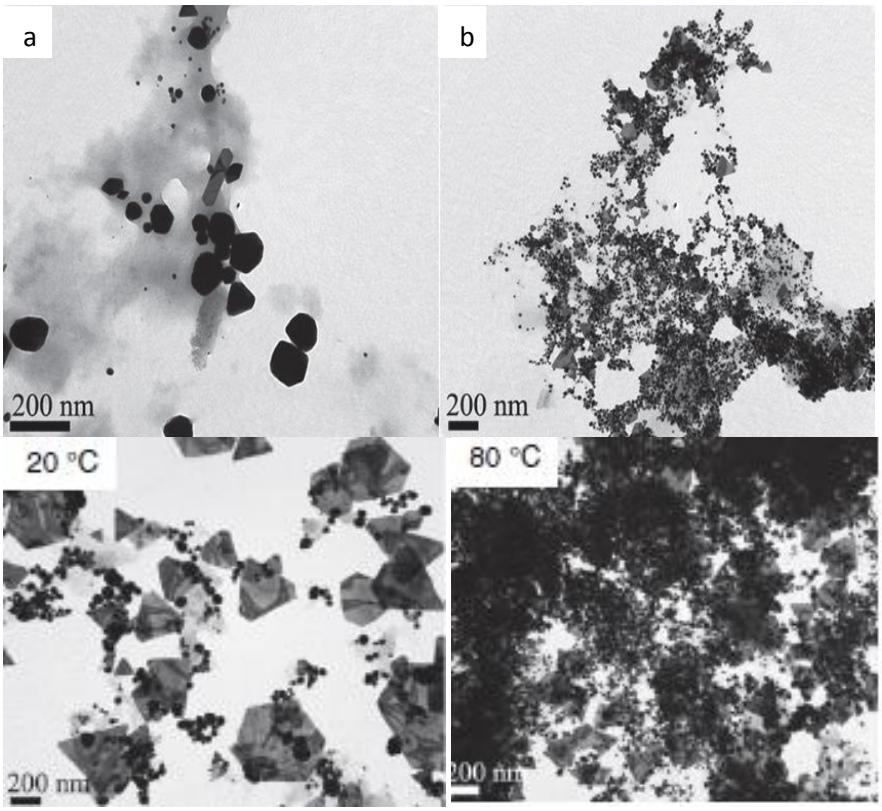

Figure 3.6: TEM images of nanoparticles at different pH (a) pH 3,(b) ph 5and temperature 20° and 80°.[8]

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The 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. As the amount of extract is increased, the stronger the interaction between the extract biomolecules and nascent nanoparticles, thus the yield of nanoparticles increased as shown by surface Plasmon resonance bands in the UV–vis–NIR spectra [9].

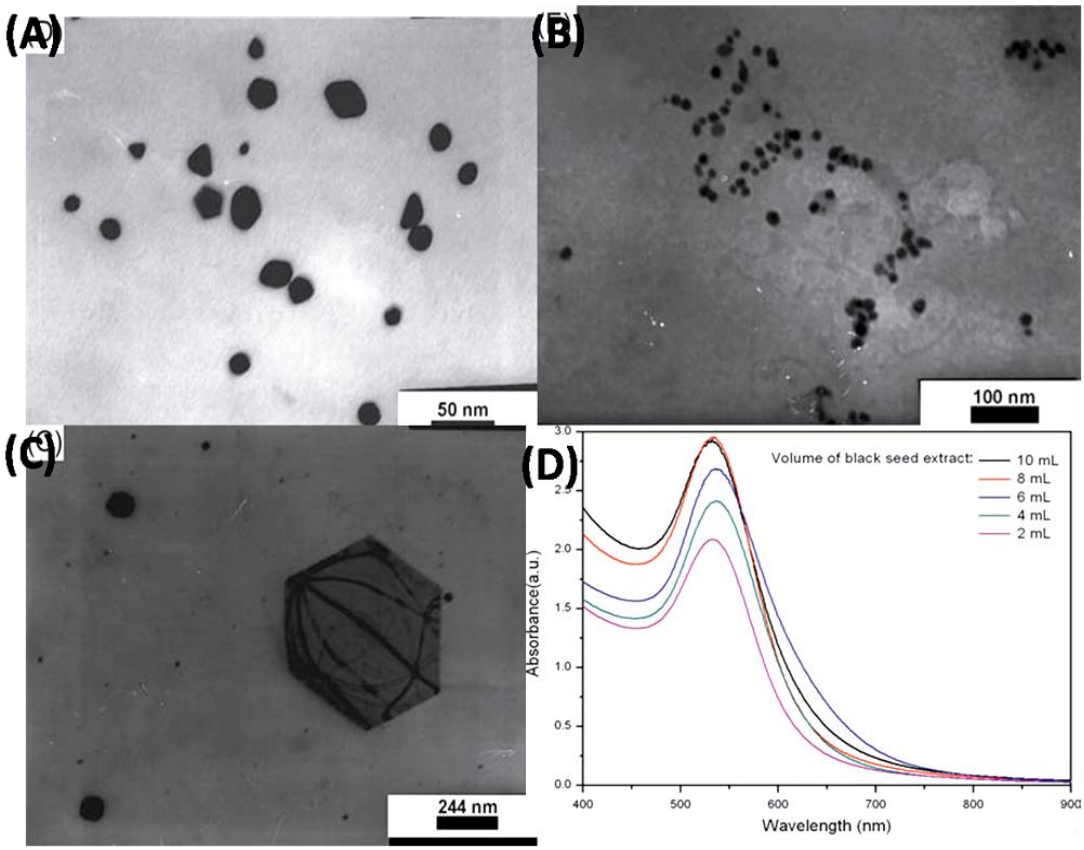


Figure 3.7: TEM images of 8ml extract (A), 10ml (B), thermal induced (C), UV spectrometer (D).[9]

Fenugreek seeds

Gold nanoparticles having different sizes in the range from 15 to 25 nm could be obtained by controlling the synthesis parameters. The nanoparticles have been characterized by UV–Visible spectroscopy, transmission electron microscopy (TEM. The high crystallinity of nanoparticles is evident from clear lattice fringes in the HRTEM images.

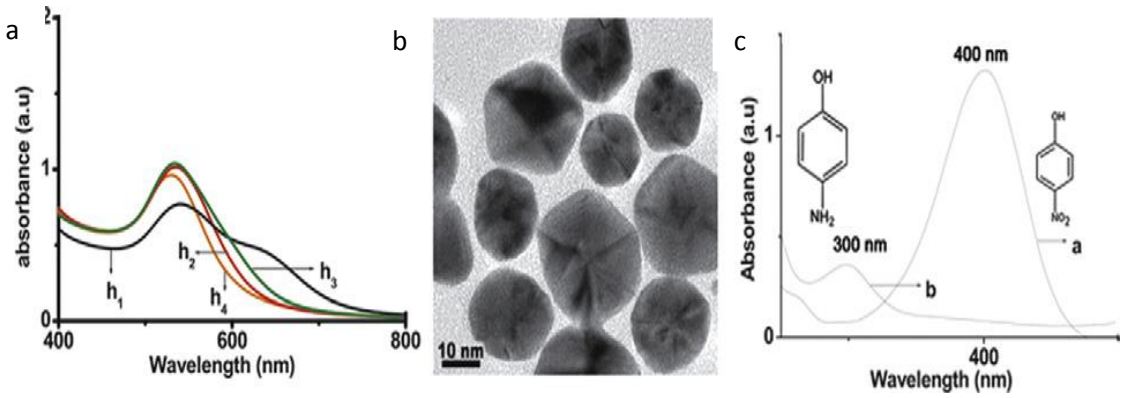
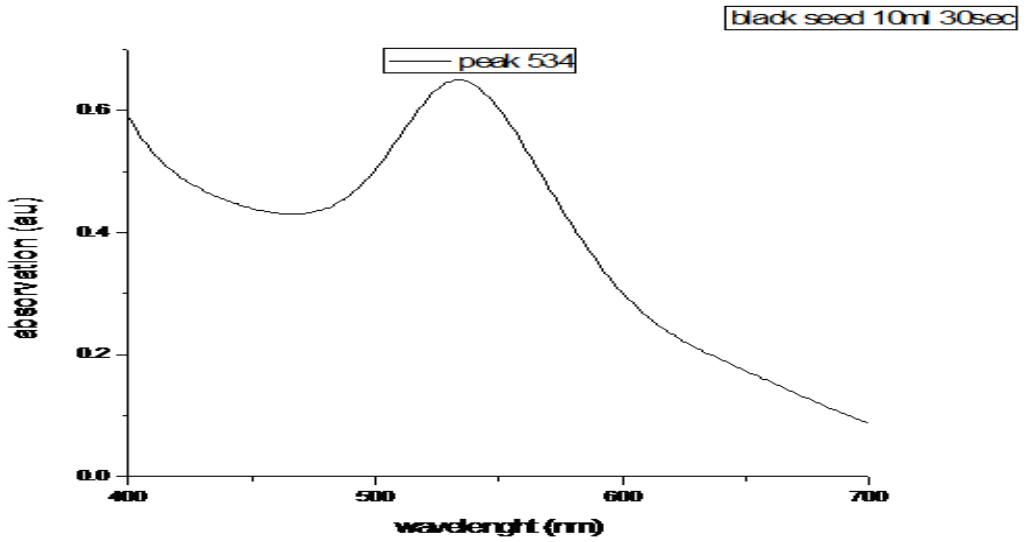


Figure 3.8: UV/visible spectroscopy (a), TEM result (b), and UV with structure (c).[10]

3.5 Results and discussion

UV/visible spectrometer

UV/visible absorption of black seed and fenugreek seed shown in below figures



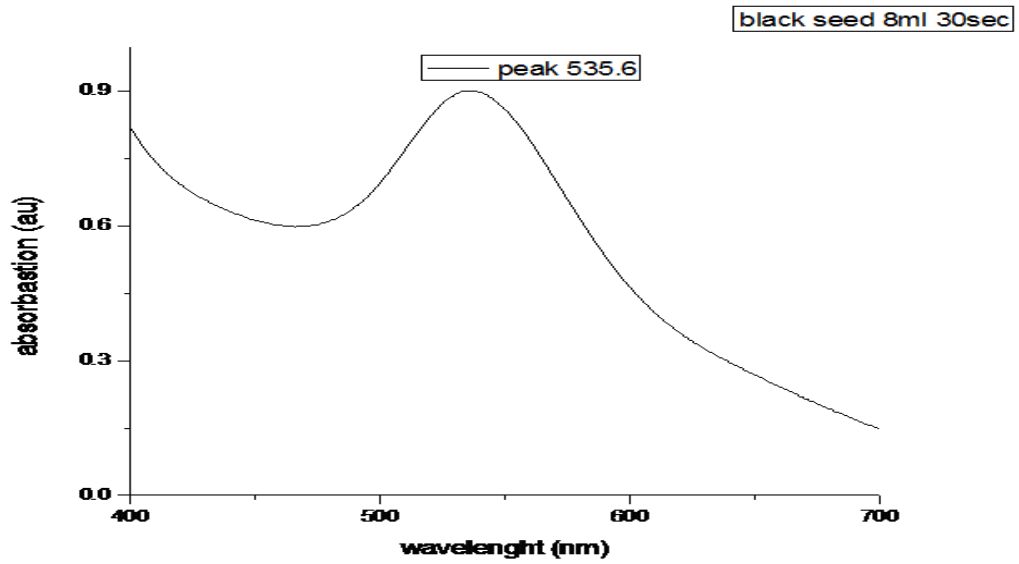


Figure 3.9: UV/visible spectrum for black seed synthesis using 8 ml extract and 10ml microwave irradiation 30 second.

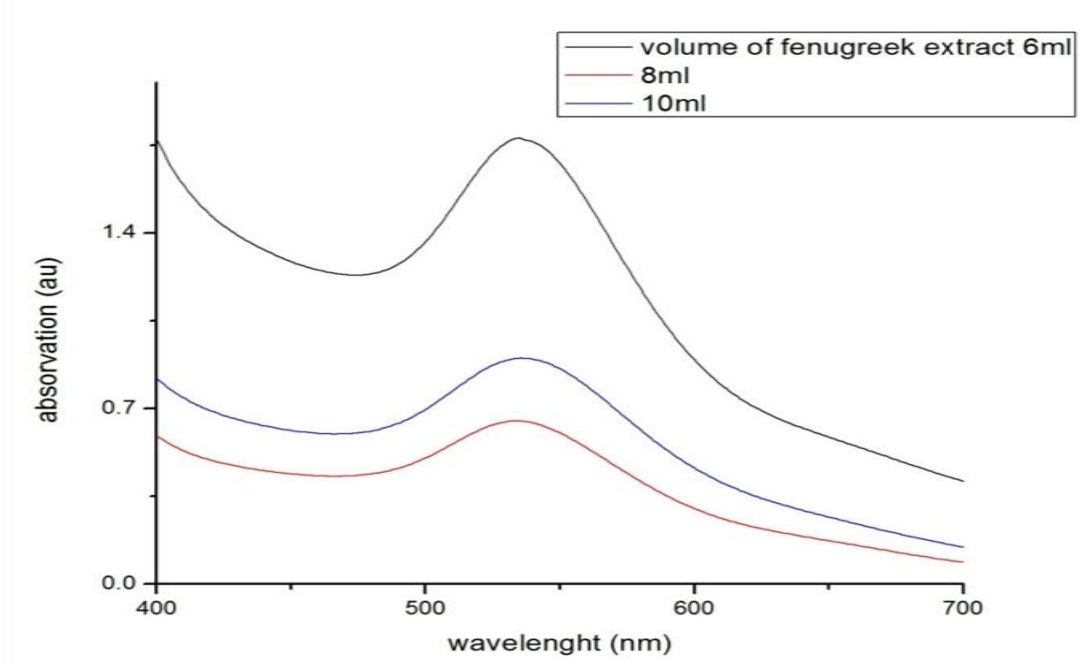


Figure 3.10: UV/visible spectrum for fenugreek seed synthesis using 6, 8, 10 ml extract by microwave 30 sec

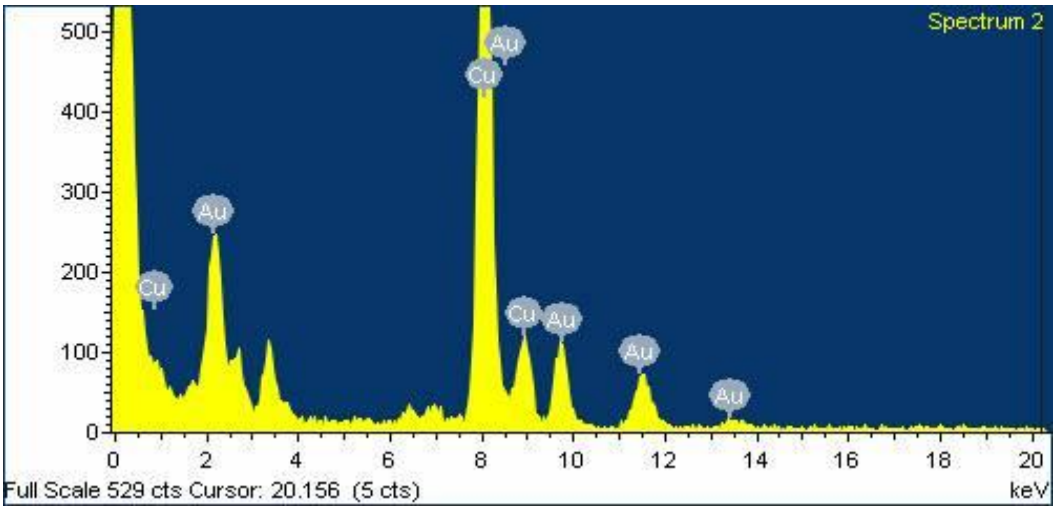
The formation of GNPs was visually confirmed from the change in color of the reaction mixture from yellow to ruby red. UV-visible spectral analysis of the reaction products exhibited sharp surface Plasmon resonance (SPR) peaks, indicating the successful formation of GNPs

The UV-Vis spectrum data represents the shift in SPR plane band towards lower wavelength when the extract increase and this is assigned to the decrease in the size nanoparticles (from 534 to536).

EDX

Figure shows the typical EDX spectrum of gold. The analysis of EDX spectra determines the percentage of Au present in the samples. From the spectrum it is clear that the gold distributed inside the nanoparticles. The presence of carbon in EDX spectra was due to the copper grid which is from the sample holder.

The analysis of EDX spectra determines the percentage of Au present in the sample Spectrums 2, 3 fenugreek extract 8, and 10 ml Spectrum 1 for black seed 10 ml.



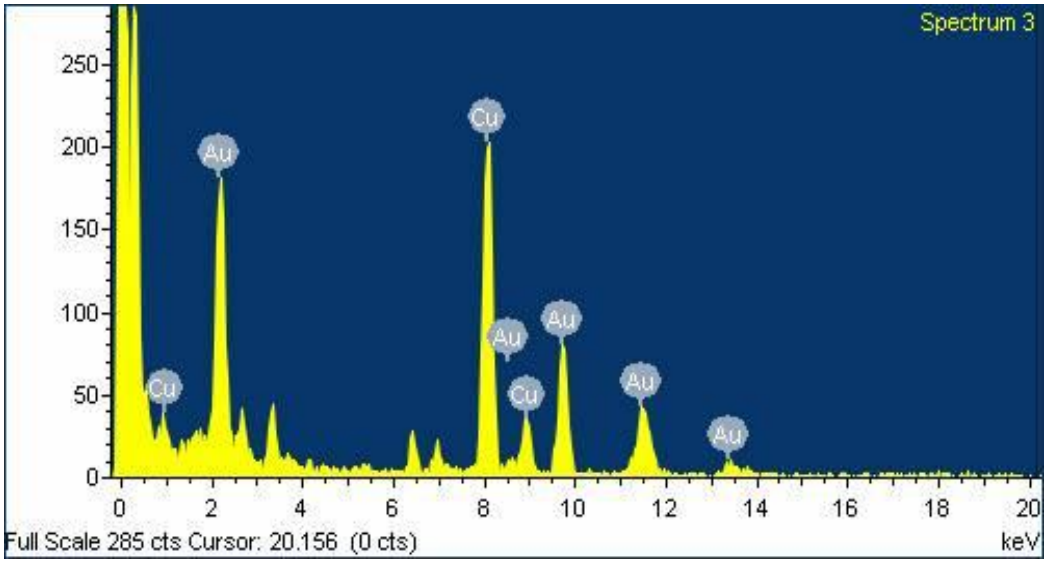


Figure 3.11: EDX spectrums of fenugreek GNPS

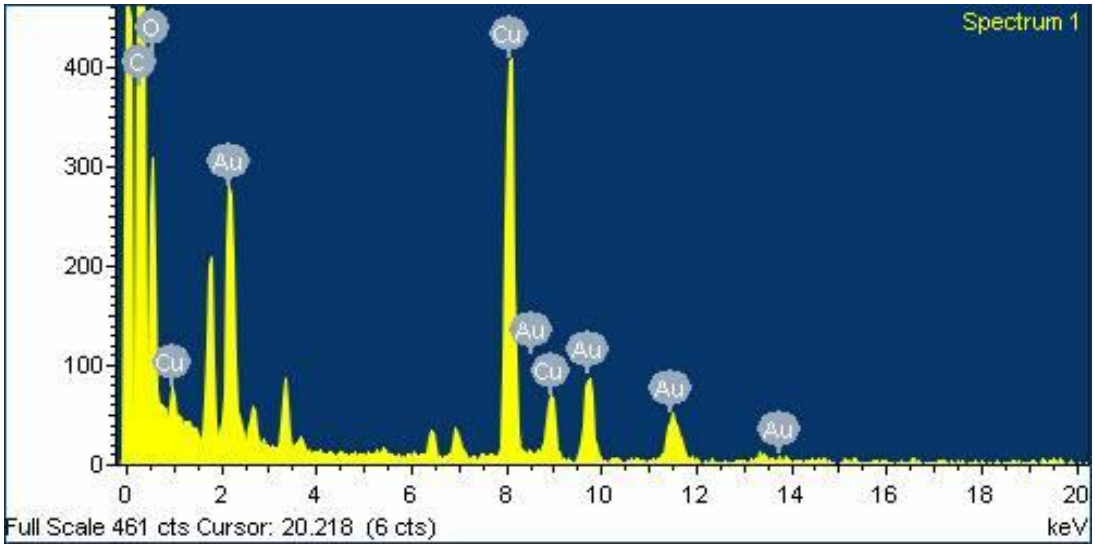


Figure 3.12: EDX spectrums of black seed GNPS.

XRD

The XRD diffraction peaks to indicate formation of gold in figure 3.11 and3.12

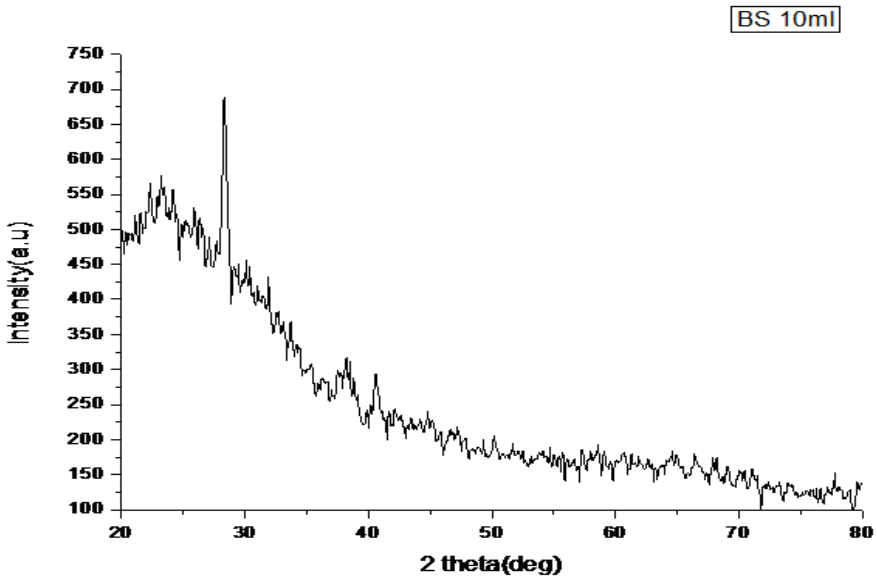
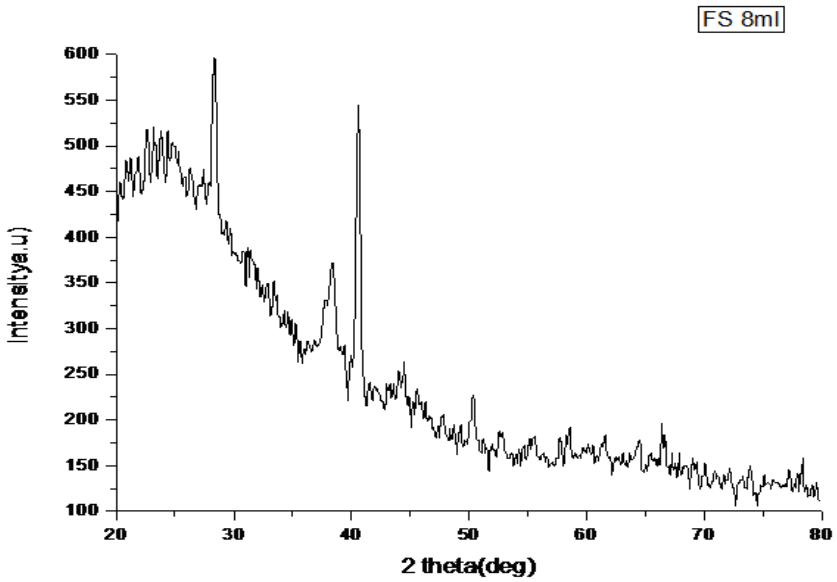


Figure 3.13: the XRD pattern of dried GNPs synthesized by black seed 10ml.



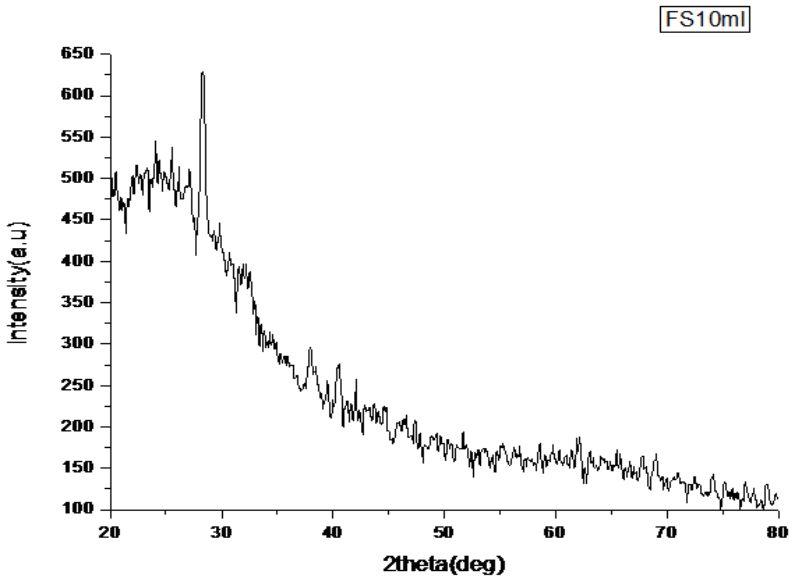


Figure 3.14: fenugreek XRD

The XRD peaks are found to be broad indicating the formation of diffraction peaks are observed, reflections of face centered structure of metallic gold, respectively revealing that the synthesized gold nanoparticles are composed of pure crystalline gold the peak corresponding to high plane is more intense than the other planes suggesting that is the predominant orientation as confirmed by the high resolution TEM measurement.

FTIR

Figure 3.13 FTIR spectrums of gold nanoparticles. The inset shows the possible mechanism of formation of gold nanoparticles.

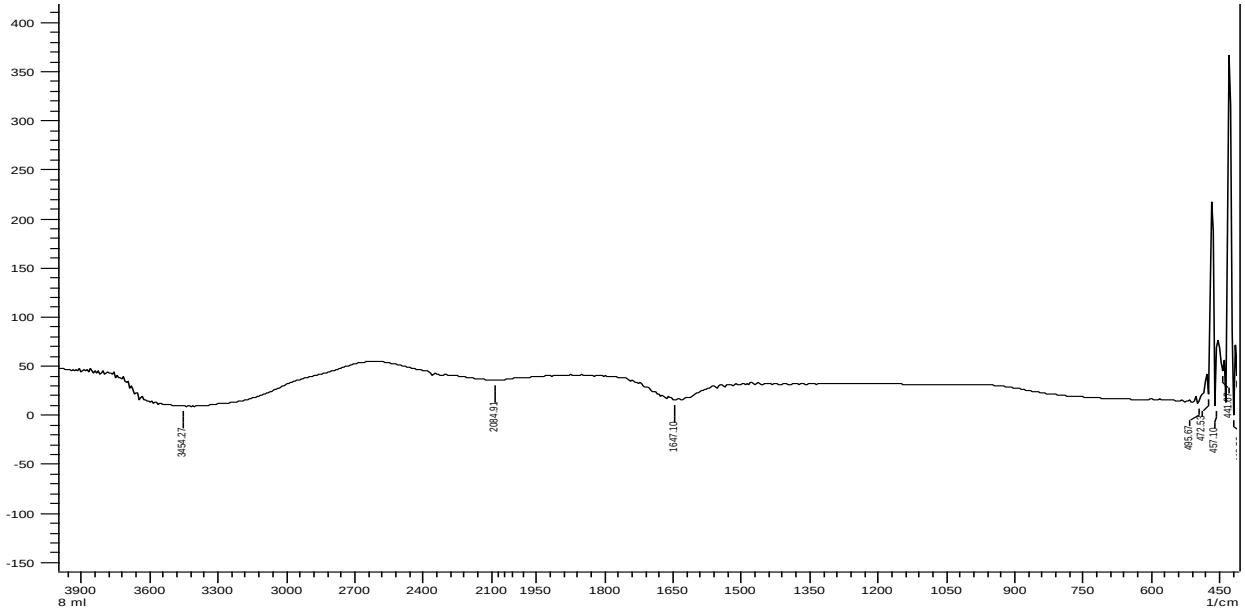


Figure 3.15: FTIR spectroscopy of GNPs prepared by 8ml fenugreek seed extract

Table 3.1: Characteristic IR bands

frequency,cm–1(range)	bond	functional group
3454.27(3500–3200 (s,b))	O–H stretch, H–bonded	alcohols, phenols
2084.91(2210–2000)	(–O–C≡N)	Cyanato
1647.1(1680–1640 (m))	–C=C– stretch	alkenes

The phytochemical analysis of the dried seed extract of fenugreek has been show the presence of proteins, vitamins, flavonoids, terpenoids, carotenoids, cumarins, curcumins, lignin, saponin and plant sterol [17]. 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.

TEM results

The morphology and the crystalline nature were further investigated by TEM images. Figures 3.16 to 3.21 shows the TEM images of fenugreek 8,10ml and black seed 10ml.

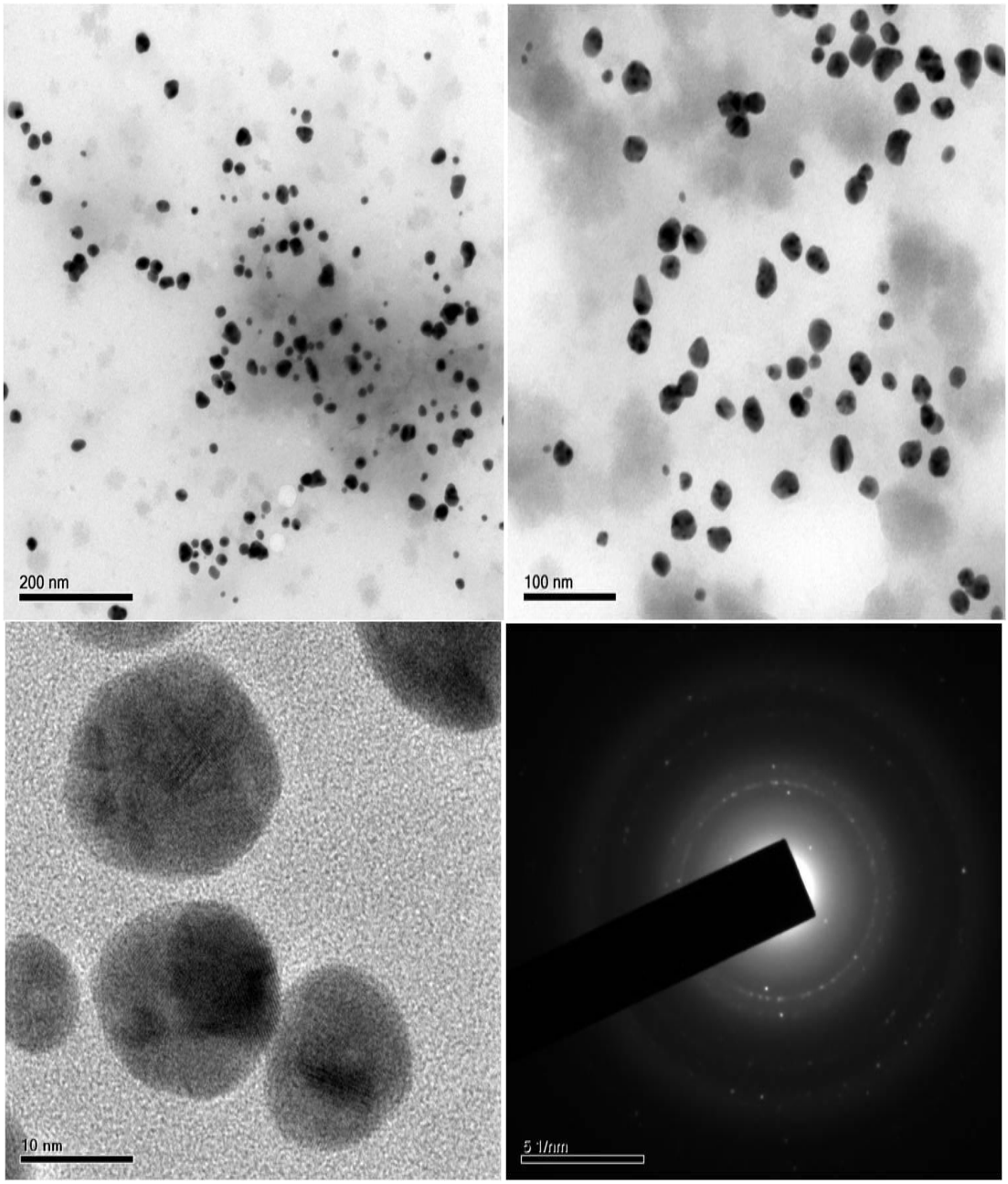


Figure 3.16: TEM images of GNPs formed by black seed extract 10ml in different scale bare and selected area diffraction pattern.

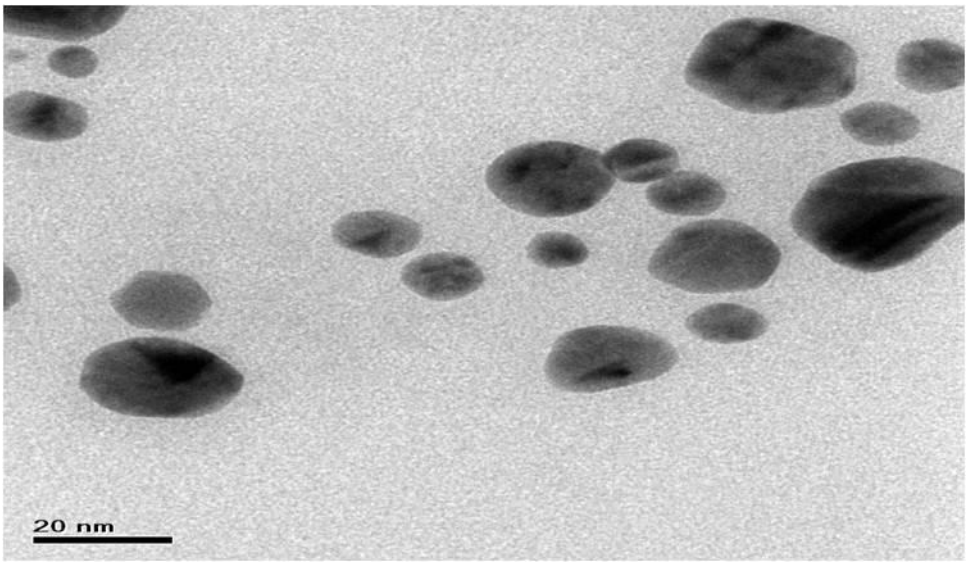


Figure 3.17: TEM image of GNPs formed by black seed extract 10ml, and its histogram of particles size distribution

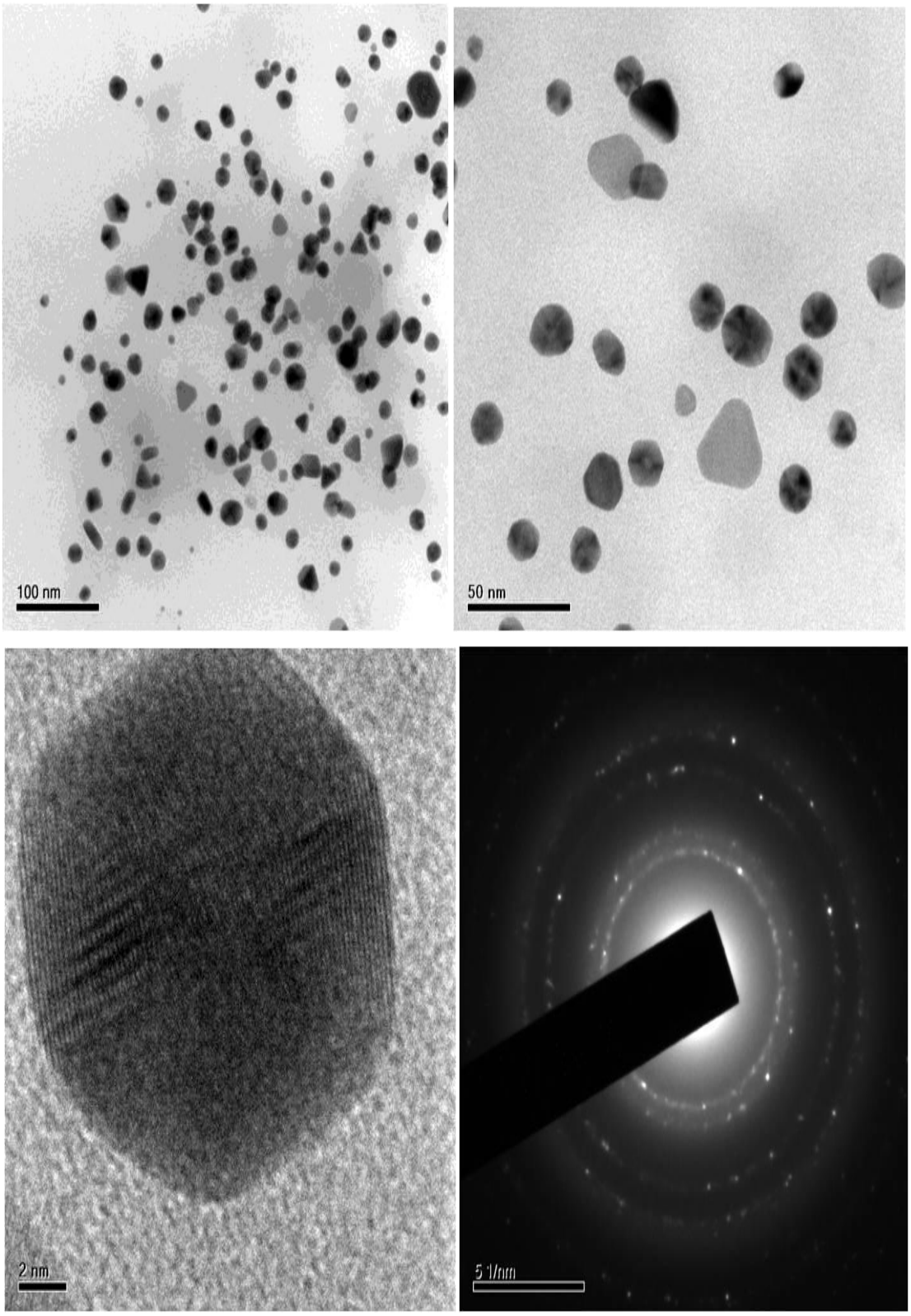


Figure 3.18: TEM images of GNPs formed by fenugreek seed extract 8ml in different scale bare and selected area diffraction pattern.

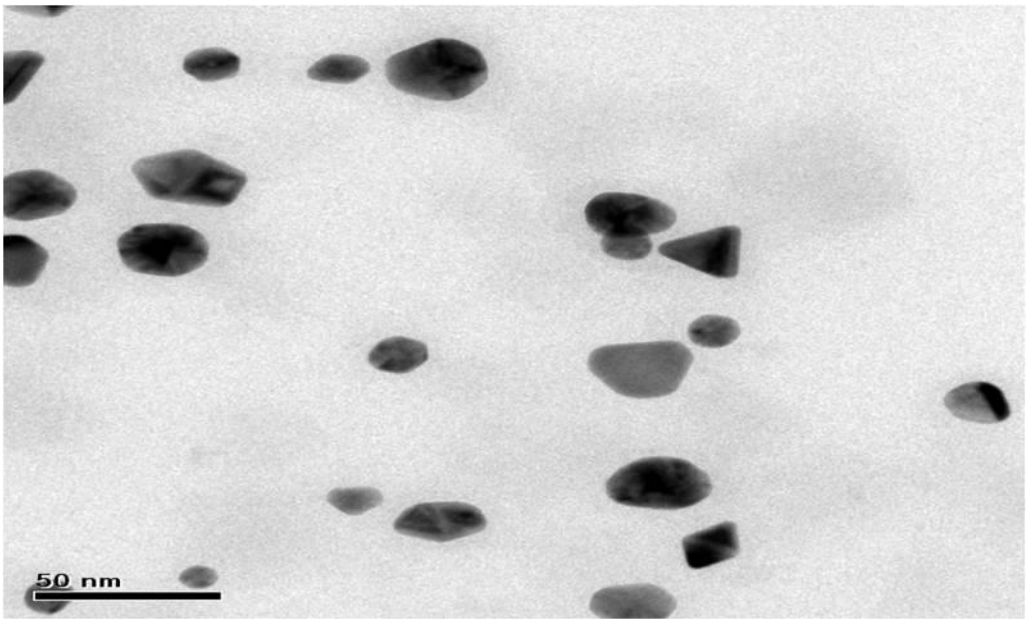


Figure 3.19: TEM image of GNPs formed by fenugreek seed extract 8ml, its histogram of particles size distribution

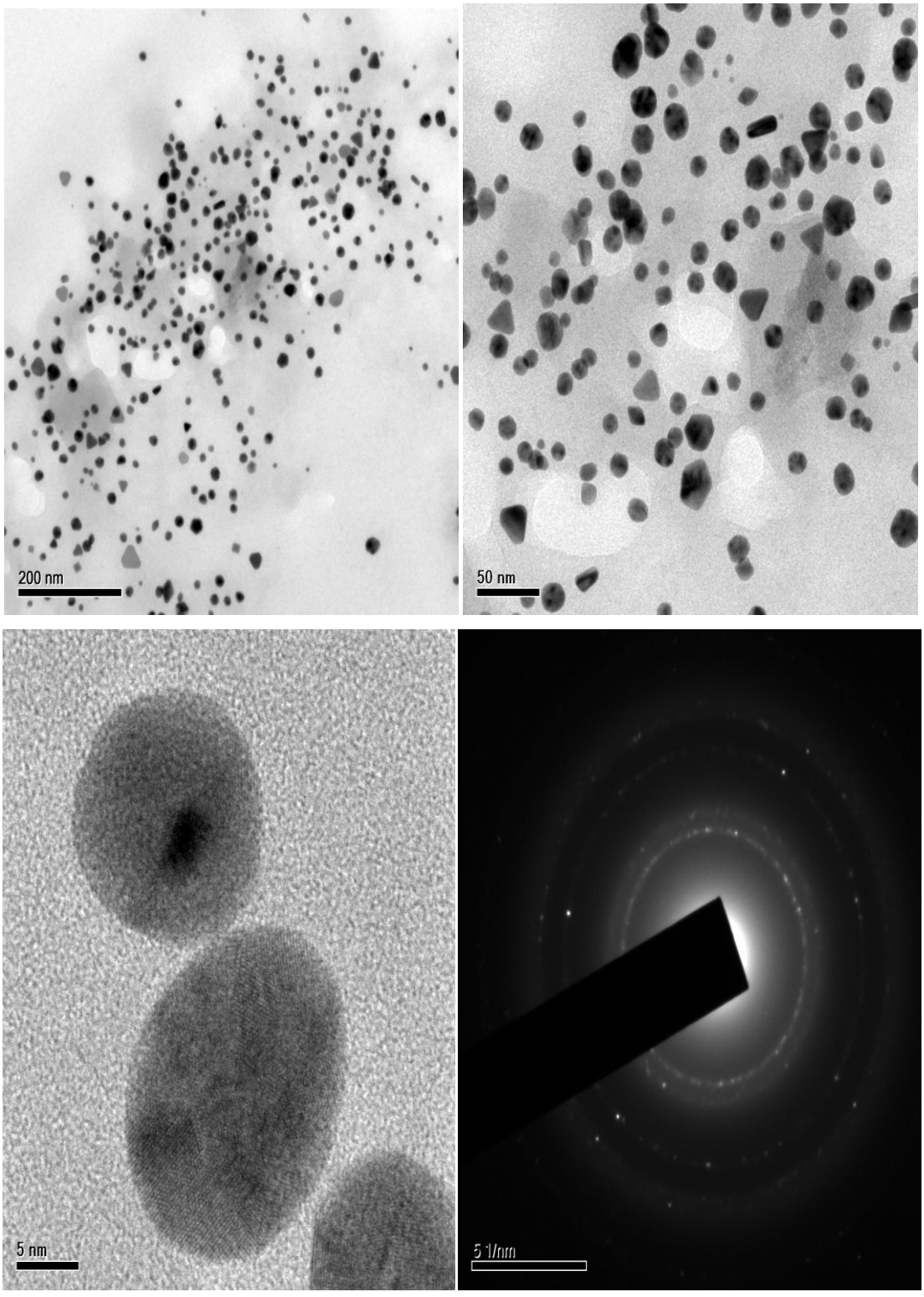


Figure 3.20: TEM images of GNPs formed by fenugreek seed extract 10ml in different scale bare and selected area diffraction pattern

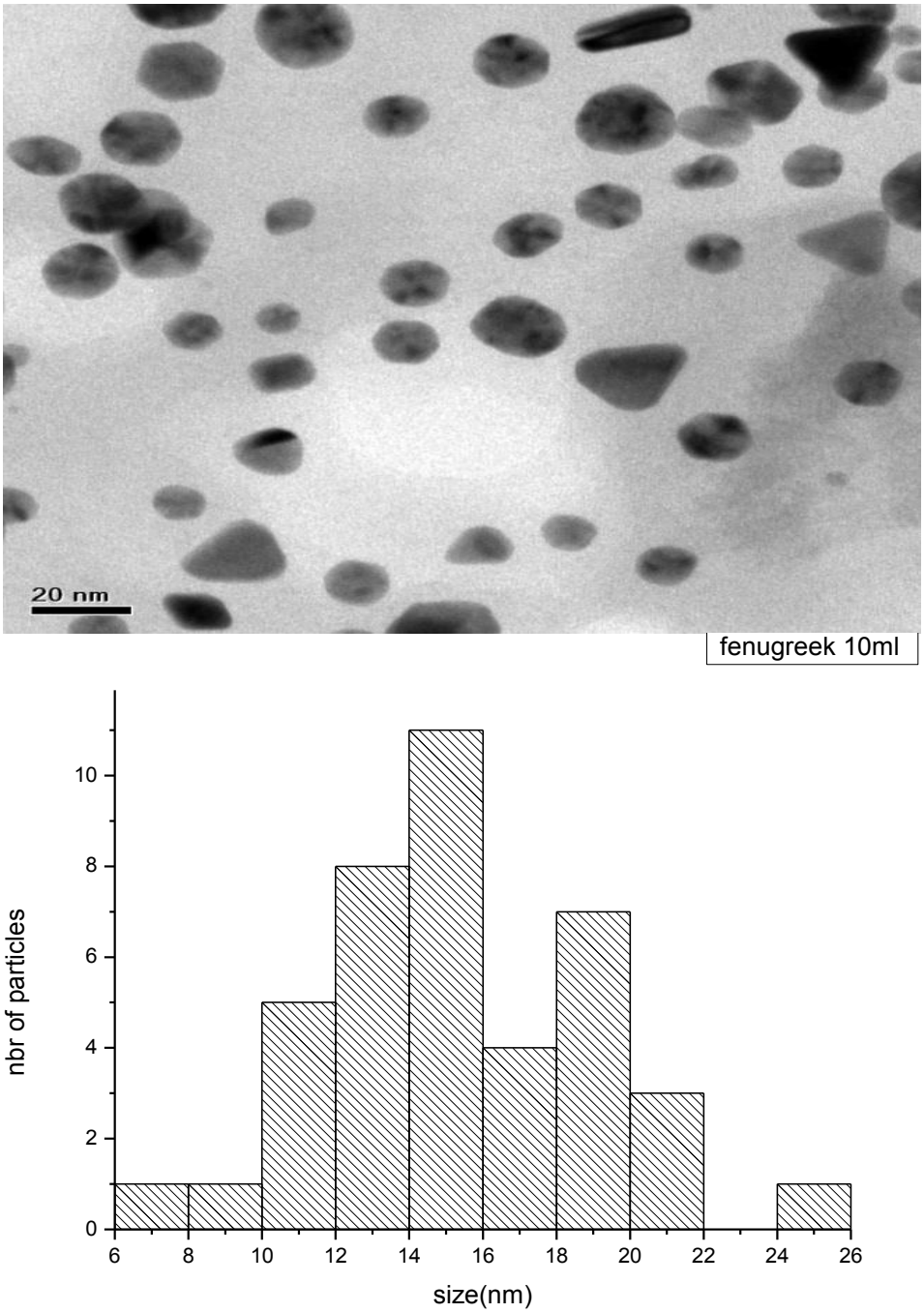


Figure 3.21: TEM image of GNPs formed by fenugreek seed extract 10ml, its histogram of particles size distribution

Typical TEM images obtained for colloids black seed 10 ml and fenugreek (10 and 8 ml) extract solution is shown in figures. From black seed images it is clear that the morphology of gold nanoparticles is almost spherical. For fenugreek seed we have different morphologies present in the samples at same concentrations. The table below summarize the result of TEM images histograms, show that the size decrease when extract increase ,for black and fenugreek seed 10ml extract approximately have the same mean.

Table 3.2: size ranges and means

Sample	Size range(nm)	mean	shapes
Black seed 10ml	9.12_28.87	15.62	almost spherical
Fenugreek 8ml	9.35_31.78	20.88	Spherical, hexagonal, triangle. rod, cubic,
Fenugreek 10ml	6.51_42.96	15.39	Spherical, hexagonal, triangle. rod, cubic,

3.6 Conclusion

Stable gold colloids having nearly spherical NPs have been synthesized using the aqueous extract of black seeds. Fenugreek seeds present different shapes that lead to wide range of applications GNPs synthesized by fenugreek extract more stable. Concentrations of extract are important factors determining the size distribution of GNPs

Chapter four

GNPs Biosensors

4.1 Introduction

Gold based nanostructures are important research subjects in nanotechnology. The profound properties of GNPs have been gradually observed as important nanostructured materials. The highly favorable properties, including the large surface to volume ratio, unique optical and electronic properties, and easy surface modification, have brought intensive focus on GNPs from both research and industry. Many efforts have been devoted to tailor the properties of GNPs for specific applications, especially in sensor development. The morphology, solubility, surface functionality and stability of GNPs can be controlled via different synthetic routes.

Most clinical analysis is carried out by specialized staff in laboratories employing desk-top instruments, thus assuring the highest possible confidence in the obtained results. However, there are many cases in which a critical clinical analysis cannot be performed in those optimal conditions because of the lack of trained analysts or the required facilities, as is often the case in underdeveloped or isolated areas. In those cases, biosensors, which are compact analytical devices for the detection of specific analytes, can be the only option to make a trustworthy medical diagnosis. Especially immunosensors, a type of biosensors aimed at the detection of the presence of specific antibodies or antigens, are particularly important for the diagnosis of diseases in remote environments, where carrying out immunoassays such as ELISA (Enzyme-Linked Immunosorbent Assay) is not an option. Although the possibility of carrying out in situ or point of care diagnosis with a minimum required training is a major reason for the development of biosensors in general and immunosensors in particular, there are many other reasons. For instance, fast, nonexpensive, multiple assays can ideally be performed with immunosensors and could be of help in epidemics to make proper diagnosis and follow the epidemic spreading.

Immunosensors make use of specific interactions between an antibody and an antigen. Antibodies are proteins generated by the immune system to identify bacteria, viruses, and parasites. The affinity between antibodies and antigens is very strong but of non-covalent nature. The development of sensitive and stable biological recognition elements is a key task in biosensors. However, biosensors, as a consequence of being

highly integrated compact devices, are at a crossroad of different fields of knowledge. Biology and biotechnology are behind the key component of biosensors, as the sensitive biological elements provide the necessary specificity for the test.

4.2 Review of test strips application

The researches and advanced application of gold nanostructures have emerged only in the recent decades. Actually, they are old materials used by ancient Chinese and Egyptians in the fifth or fourth century B.C **[18]** there is also evidences showing that ancient Romans used gold colloids to stain glass red or mauve. However, the first scientific literature of gold nanoparticles (GNPS) was reported by Michael Faraday in the 1850s [19**].**

The development of the rapid, immunochromatographic test strip, also known as lateral flow immunoassay (LFIA), is the result of convergence of several threads that can be traced back to the 1950s. However, the concept of rapid diagnostic tests based on body fluids dated back significantly further [24] Documented evidence of saliva- and urine-based diagnostics existed several thousand years ago.

The technical basis of the lateral flow immunoassay was derived from the latex agglutination assay, the first of which was developed in 1956 by Plotz and Singer [25]. In the same period, plate-based immunoassays were being developed**.** The main application driving the early development of the solid phase, rapid-test technology was the human pregnancy test, which represented continual historical interest in urine testing for medical diagnostic purposes. This particular testing application made great strides in the 1970s, as a result of improvements in antibody generation technologies and significant gains in understanding of the biology and detection of human chorionic gonadotropin (hCG), derived largely from the work performed by Vaitukaitis and co-workers [26]. However, to fully develop the lateral flow test platform, a variety of other enabling technologies were also required. These include technologies as diverse as nitrocellulose membrane manufacturing, antibody generation, fluid dispensing and processing equipment, as well as the evolution of a bank of knowledge in development and manufacturing methodologies. All of these elements were required to render a mélange of complex chemicals, biologicals,

papers, polymers, people, and processes into a simple and easy-to-use test, which is able to adequately perform to provide prognostic results in a variety of critical applications. Many of these facilitative technologies had evolved throughout the early

1990s, to the point where many are now mature, off-the shelf technologies. As a result of the early work in all of these areas, the first lateral flow products were introduced to the market in the late 1980s. Since then, the technology, its applications, and the industry have all continued to evolve. As of 2006, over 200 companies worldwide are producing a range of testing format. The application of the technology has expanded well beyond clinical diagnostics to areas as diverse as veterinary, agriculture, biowarfare, food, environmental health and safety, industrial testing, as well as newer areas such as molecular diagnostics and theranostics.

4.3 Universal immunochromatography Assay (ICT)

Lateral flow assays (LFA) or (ICT) are an inexpensive immunological technology that offers specific and fast result. It is particularly useful in the area of point-of care (POC) diagnostics, which eliminates the need for laboratory work conducted by trained personnel and in specialized facilities. ICT tests can be used to detect analytes in a liquid sample, such as urine, human serum, plasma, or whole blood, which can be applied to the device in a single step. The analyte of interest could be either a low-molecular-mass analyte such as drug residue, antibiotics, hormone, or a high-molecular-mass molecule such as proteins. In general, LFAs make use of a porous membrane, usually made out of nitrocellulose that is highly adsorptive for proteins. Various types of antibodies or ligands can then be immobilized in the capture zone of the membrane either directly or indirectly, depending on the nature of the reaction membrane. The liquid sample, is then mixed with a buffer solution and labeling agents, which could be latex beads, colloidal gold particles, dye-encapsulating liposomes, carbon black, silica, etc. The mixture is then allowed to migrate towards the capture zone by capillary forces. The analytes and/or the labeling agents are then captured by the immobilized antibodies

lmmunoassays become important when

1) Fast measurement and evaluation are required

2) Highest possible detection strength is required

3) Large numbers of samples are to be expected

4) Only complex and expensive analytical methods are otherwise available

4.3.1 Overall Design

A typical biosensor is composed of a reaction membrane, usually made out of nitrocellulose, a sample pad, a conjugate pad, and an absorbent pad. The conjugate pad, commonly made out of glass fiber filter, paper filters, and surface-treated (hydrophilic) propylene filters, is used to store and deliver the detector agent, which are dye encapsulating liposomes in this case. The absorbent pad, which is placed at the end of the test strip, serves as the sink for the sample as it migrates through the strip. The absorbent pad is often made out of cellulosic paper. It works to enhance the assay sensitivity by increasing the total amount of volume of sample that can be accommodated on the membrane. Lastly, the sample pad, which will be placed at the beginning of the test strip, acts as a filtration device by removing undesirable fractions of the liquid sample. It also serves to absorb the sample and provide a uniform flow on the test strip. For high viscosity and high concentration of particles in fluid, such as whole blood samples, special blood separation filters should be used.

4.3.2 Basic Principle to produce ICT

The basic principle to produce biosensor is change in the optical absorption of GNPs [30]. The basic lateral flow assay consists of six different parts.

(1), the sample is added to the sample pad from which it continues to the conjugate pad

(2), where depending on the assay type the labeled antibody or antigen is immobilized. Then the solution is moving in the membrane

(3) By capillary forces. When the solution reaches the test line

(4) The antibodies there attach to antigens. Usually after the test line there is also a control line

(5) Where excess antibodies can bind with other antibodies. The function of this site is to control that the test and the capillary flow are functional.

(6) In the opposite end of the strip when compared to the sample pad is the sixth part, the absorbent pad which absorbs excess liquid. The principle and construction of lateral flow assay is presented in Figure (4.1).

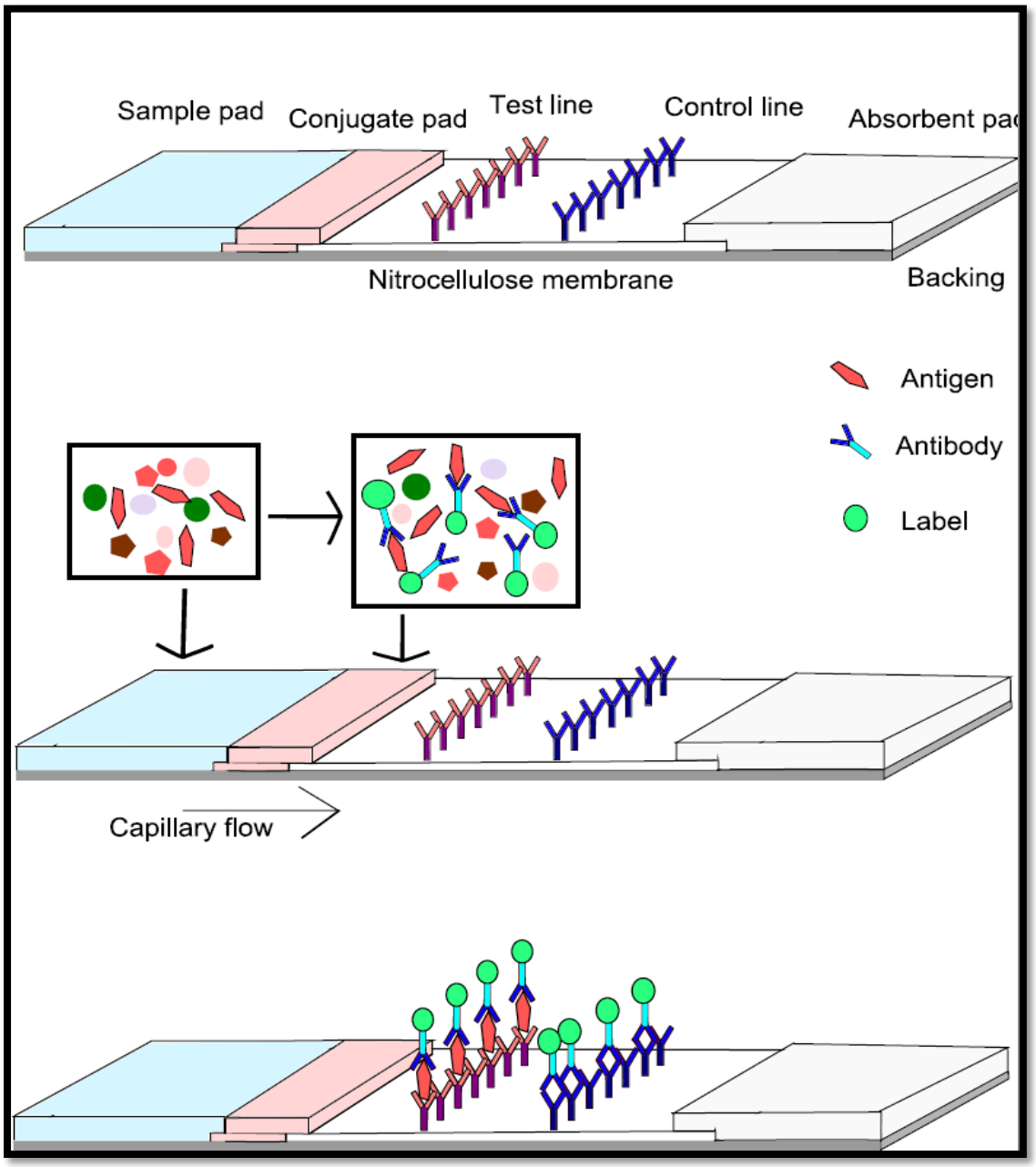


Figure 4.1: The principle of lateral flow strip presented with sandwich assay. Sample solution is added to sample pad. Antigen attaches to antibodies in conjugate pad and the complex formed attach to test line antibodies. The excess labeled antibodies bind with antibodies in control line.[21]

4.3.3 Common ICT types

First Direct immunoassay is a method where the label is added to the solution of the antigen. The labeled antigen binds with the antibody which is immobilized on the surface. After washing the excess label away the bound labels can be detected.

Second method is competitive assay. The competitive format, the labeling agent is also coated with or conjugated to antibodies to the analyte of interest. But the analyte of interest, instead of the antibody, is immobilized on the membranes. As the liquid mixture reach the capture zone, analyte in the sample, and then compete with the immobilized analytes for the binding sites on the antibodies tagged with the labeling agent. The absence of signal will then be an indication of a sufficient amount of analytes present in the sample. In this system the known amount of antigen is labeled in advance and added to another solution of the same antigens which concentration is wanted to measure. When this solution is then incubated with the surface antibodies, labeled and unlabeled antigens will compete for the binding sites and attach with same ratio as they are present in the solution. The amount of labeled antigens can be then determined and the concentration of unlabeled antibodies can be calculated.

The third approach is sandwich assay. In this assay format, the analyte are sandwiched between the antibody immobilized on the test membrane and a capture reagent, often another antibody, which is tagged with labeling agents to produce a signal which is indicative of the presence of the target analytes this method comprises of two antibodies which both can bind with antigen. The first antibody is attached to the surface and it captures the antigen whose concentration is to be measured. After incubation with the antigen solution the second antibody is added. This procedure differs from the others because this time the second antibody is labeled instead of the antigen. The name of the procedure is due to the antibody-antigen-antibody –complex formed in the last step. The label in the second antibody can be then measured and it directly represents the amount of antigen.[21] A sandwich assay, or direct assay format, is often used for the detection of high-molecular-mass analyte such as proteins.

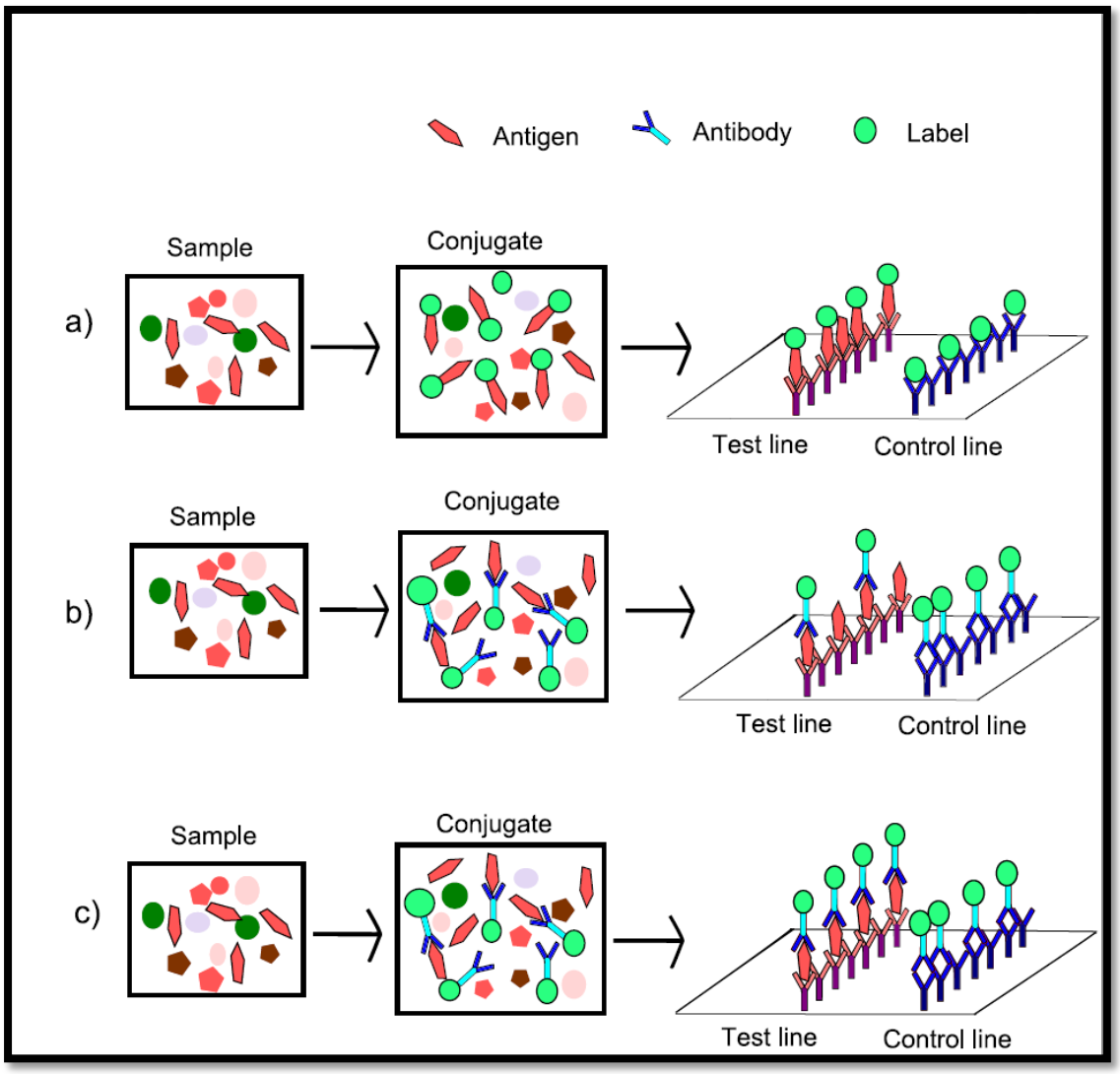


Figure 4.2: Common immunoassay procedures in lateral flow assay: a) Direct immunoassay, b) competitive immunoassay, c) sandwich immunoassay.[21]

4.4 Properties of Antibodies

In biosensor applications, basically three types of antibodies can be used: monoclonal, polyclonal and recombinant antibodies [22]. The division is based on how they are produced. Polyclonal antibodies are isolated from the whole serum after immunization and they can recognize many different epitopes of the antigen. Monoclonal antibodies, in turn, are produced only by one hybridoma cell. They recognize always the same epitope of the antigen and they are exact copies of each other. Recombinant antibodies may not be whole antibodies but they have the

essential Fab-parts. They are produced through recombinant DNA technique, where the DNA, coding the wanted parts of the antibody, is introduced to bacteria which then produce those parts. Antibodies or Fabs are then extracted from the bacteria. [23].

In order to use antibodies in lateral flow assay they have to be purified properly. Monoclonal antibodies serve the best because then two antibodies detecting antigen from different angles can be used in conjugate and capturing. The consistency of the antibody solution is important, as well as the stability of antibodies after the membrane is dried

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4.5 Assay components and materials

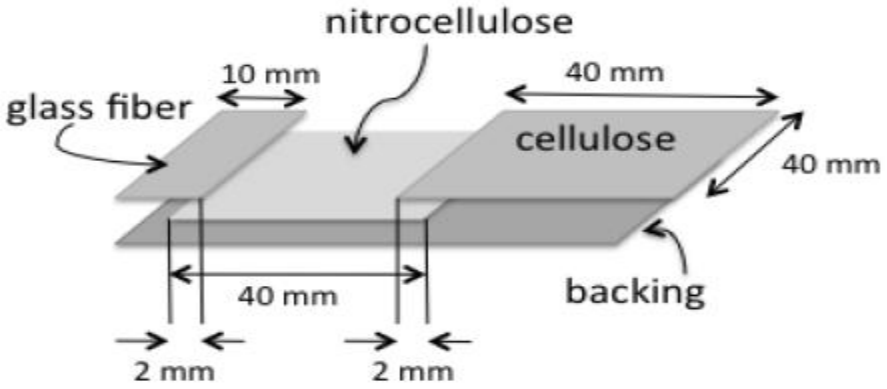

Figure 4.3: Over lapping between assay components.[34]

Table 4.1: Assay components function, materials and treatments.[33]

Component	Function	Material	Pretreatment
Sample pad	Modulate any chemical Variability in the sample, treat the crude sample and release the assay adjusted sample	100% cotton linter, glass fiber, rayon and filtration materials	Impregnation with pH buffer, surfactants, blocking reagents, additives and drying
Conjugate pad	Couple the analyte in the sample with the conjugate and	Glass fibers, polyesters, and rayon	Immersion of the pad in aqueous solutions (e.g. proteins,

	release the sample		surfactants, polymers), drying at high temperatures
Membrane	Facilitate as a capturing mechanism and form visible bands when analyte is present/absent	Nitrocellulose, nylon and polyvinylidene fluoride	Rewetting agents (i.e. surfactants), electrostatic attraction to bind proteins, deposition of test and control lines, drying at elevated temperature, blocking
Absorption pad	Serve as the sink for excessive sample and prevent wicking towards the reaction membrane	100% cotton linter, high-density cellulose	-
Backing	Keep all the components in one place, provide rigidity and facilitate easy handling	Polystyrene, vinyl (poly vinyl chloride or PVC), and polyester	Lamination and cutting
Adhesive	Hold the assay components in place and be compatible with proteins during storage	Diagnostic grade medium tack adhesives (e.g. GL187 pressure-sensitive adhesive)	-
Cassette	Preserve the assay from environmental conditions and prevent contamination, expose the sample pad, maintain the alignment of components, indicate the position of test and control lines	Low-cost plastics	-

4.6 Colloidal Gold in ICT.

Colloidal gold is used as a label in lateral flow assay without any complex signal amplification procedure, and it serves as a reference method to magnetic particles due to its widespread use in commercial products as well as in research. The reasons for its popularity are that it is easy to use and the procedure has only one phase before the signal can be detected. Additionally, the results can be read without a reading device.

Gold nanoparticles (with wavelengths around 510–530 nm for Au nanoparticles of around 4–40 nm diameter) that can be used for sensing. The binding of molecules to the particle surface can change the plasmon resonance frequency directly.

1-For GNPs the most feature is its red color when applied to the lateral flow test. The red colour forms the recognizable line when interpreting the results and it also shows that after the conjugation with antibodies the colloidal gold particle solution is still stable. If the gold particles form larger aggregates or settle, the flow in the membrane can be disturbed.

2-Another feature related to this work is the behavior of colloidal dispersions. Similar to fog and mist, the solution of gold nanoparticles is colloidal dispersion, where the dispersed, solid phase is kept suspended thanks to the thermal or Brownian motion.

Both the presented properties are important.

4.7 materials and method

Lateral flow strips were prepared by using gold nanotechnology and rapid test strips device technique. Colloidal gold nanoparticles were prepared by previous method. Test line is specific antigen and sample pad and control line contain different antibodies.

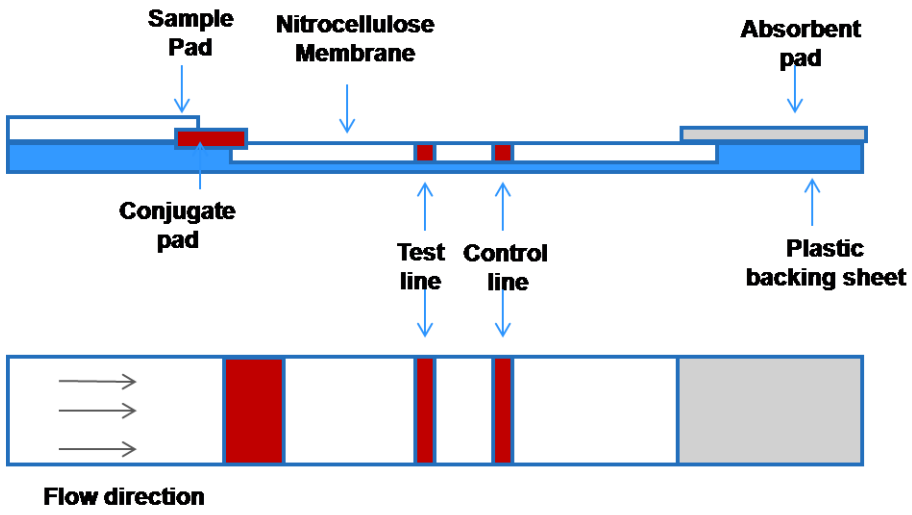

Figure 4.4: Model of the lateral flow assay used in this study. The top schematic is the side view of a test strip. The bottom schematic is the top view of a test strip

LFIA bioreactors mathematically

We consider a lateral flow (LF)

1-reactor that consists of a flat, porous membrane.

2- A sample containing the target analytes (various target analytes A.), reporter particles (P), and buffer solution is introduced in a reservoir that is in contact with a dry porous membrane (typically made of nitrocellulose).

3-The solution flows through the membrane by capillary action. Ligands(R) are immobilized at various locations in the membrane.

4-The membrane may contain many capture zones; each designed to bind specifically with a target analyte. As the solution passes through the capture zones, both analytes bound to reporters and free analytes interact with the ligands. Subsequent to the binding process, the concentration of reporter particles is measured as a function of location. When specific target analytes are present in the solution, the concentration of reporter particles and the signal generated at the corresponding capture sites will be higher than away from the capture zone. Since the locations of the capture sites are known, one can determine whether the specific target analytes are present in the sample.

Ideally, the signal's magnitude would be proportional to the target analyte's

We assume that the solution is dilute the analytes interact with the reporter particles to form particle-analyte complexes, A+ P ↔PA.

(PA) interact with the immobilized ligands of type (R) to form the complexes

RA (A+R↔RA) and RPA (PA+R↔RPA), additionally, unbound particles (P) may bind to the complex (RA) to form the Complex RPA (P+RA↔RPA).

The concentrations are functions of space and time [A] (**x**, t), where **x** is the space coordinate and t is time.

The rate of formation (FPA) of the particle-analyte complex (PA) is proportional to the product of the free analytes ([A]) and free particles ([P]).

$$\mathrm{FPA} = \mathrm{k_{a1}}[\mathrm{A}][\mathrm{P}] - \mathrm{k_{d1}}[\mathrm{PA}] \quad (4.1)$$

The rate of formation (FRA) of the ligand-analyte complex (RA) is:

$$\mathrm{FRA} = \mathrm{k_{a2}}[\mathrm{A}]([\mathrm{R_0}] - [\mathrm{RA}] - [\mathrm{RPA}]) - \mathrm{k_{d2}}[\mathrm{RA}] - \mathrm{k_{a4}}[\mathrm{RA}][\mathrm{P}] + \mathrm{k_{d4}}[\mathrm{RPA}] \quad (4.2)$$

$$\mathrm{FRPA} = \mathrm{F^1}_{\mathrm{RPA}} + \mathrm{F^2}_{\mathrm{RPA}}. \quad (4.3)$$

$$\mathrm{F^1}_{\mathrm{RPA}} = \mathrm{k_{a3}}[\mathrm{PA}]([\mathrm{R_0}] - [\mathrm{RA}] - [\mathrm{RPA}]) - \mathrm{k_{d3}}[\mathrm{RPA}](4)$$

$$F^2{}_{RPA} = k_{a4}[RA][P] - k_{d3}[RPA] \quad (4,5)$$

$$\frac{\partial[A]}{\partial t} = DA\frac{\partial^2[A]}{\partial x^2} - U\frac{\partial[A]}{\partial x} - (FPA + FRA) \quad (4.6)$$

$$\frac{\partial[PA]}{\partial t} = DP\frac{\partial^2[PA]}{\partial x^2} - U\frac{\partial[PA]}{\partial x} - (FP - F^1{}_{RPA}) \quad (4.7)$$

$$\frac{\partial[P]}{\partial t} = DP\frac{\partial^2[P]}{\partial x^2} - U\frac{\partial[P]}{\partial x} - (FP + F^2{}_{RPA}) \quad (4.8)$$

$$\frac{\partial[RA]}{\partial t} = FRA \quad (4.9)$$

$$\frac{\partial[RPA]}{\partial t} = FRPA \quad (4.10)$$

Since these conditions may vary from one case to another, we consider here two extreme cases. First instance, we assume that the mixture of the target analyte and reporter particles was allowed sufficient time to equilibrate prior to entering the membrane. This case described by following equations, the symbol e denote to equilibrium.

$$PA_e = .5[A_0] + [P_0](+\frac{k_{d1}}{k_{a1}} - \sqrt{([A_0] + [P_0]) + \frac{k_{d1}}{k_{a1}})^2 - 4[A_0] + [P_0]} \quad (4.11)$$

$$A_e = A_0 - PA_e \quad (4.12)$$

$$P_e = P - PA_e \quad (4.13)$$

$$[RPA] = \frac{k_{a3}k_{d2}[R_0][PA_e]}{(k_{d2(k_{d3}+k_{a3}}[PA_e]) + k_{a3}k_{d3}[A_e]} \quad (4.14)$$

$$[RA] = \frac{k_{a2}k_{d3}[R_0][A_e]}{(k_{d2(k_{d3}+k_{a3}}[PA_e]) + k_{a3}k_{d3}[A_e]} \quad (4.15)$$

Away from the capture zone, [RPA] = [RA] =0.

In the capture zone, the signal is proportional to S= [P] + [PA] + [RPA]. Away from the capture zone, the signal is proportional to S0= [P] + [PA] = $[P_0]$. Where S0 is background signal and define the amplitude ΔS=S-S0 and the contrast index DS=$(S-S_0)/S_0 \equiv [RPA]/[P_0]$.

When the reporter particle concentration is relatively small, DS appears to be nearly independent of [P0]. In other words, DS appears as a horizontal line. This behavior can be attributed to both S and S0 increasing at a similar rate as [P0] increases. Once the reporter particle concentration exceeds a critical value, further increases in [P0] lead to a decrease in the contrast index DS.

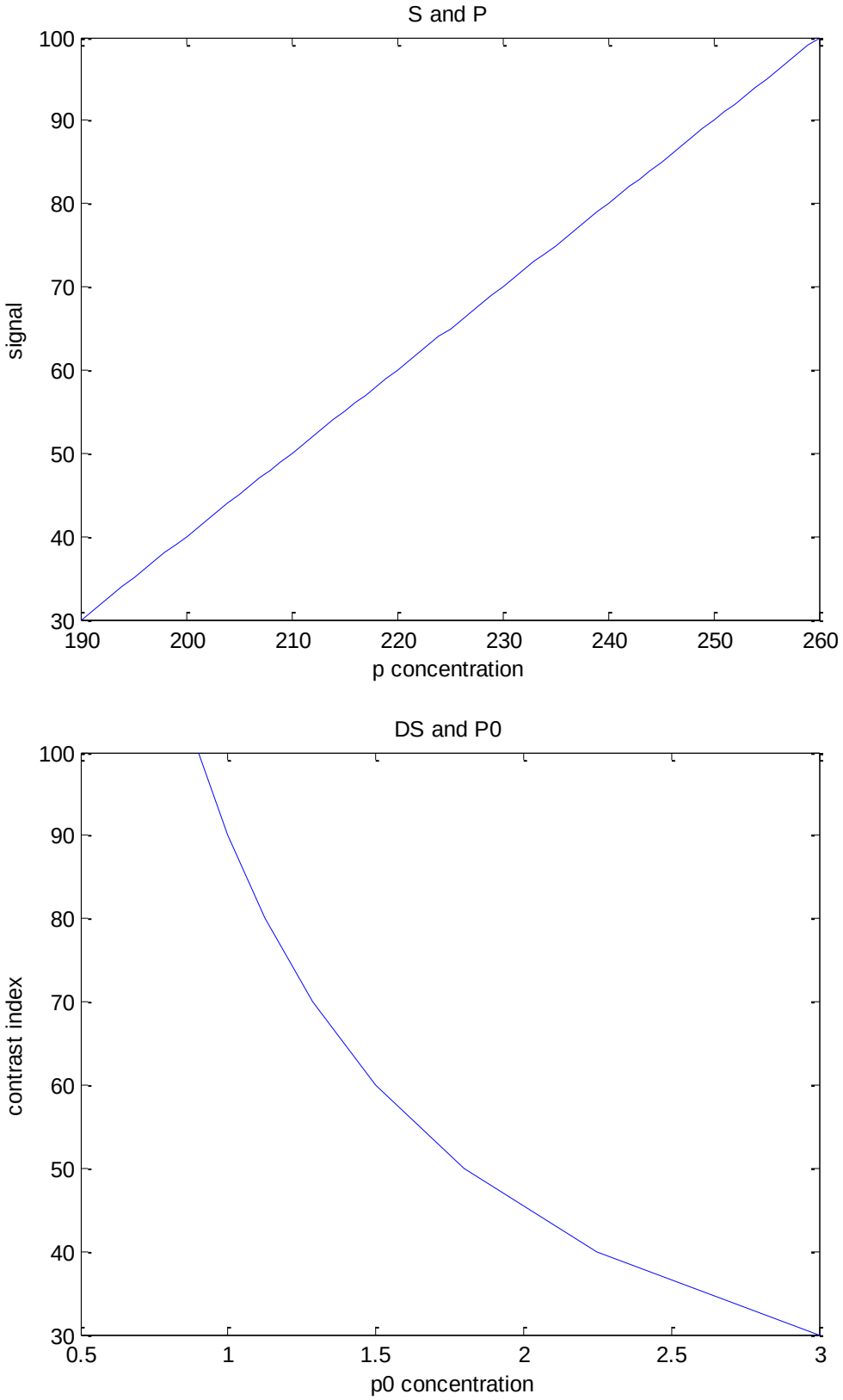

Figure 4.5: The top plot is the relation between particles concentration and the signal. The bottom plot between initial particles concentration and contrast index.

4.8 Conclusion

ICT technology is evolving rapidly. Novel approaches driven by market needs are leading to improvements in performance and utility to a vast array of new application areas. With the integration of new reading, labeling, sample-handling, and device designs comes a requirement for a new approach to system development and manufacturing. The development of highly sensitive and reproducible/quantitative next-generation point-of-need diagnostic assays requires a different, more multidisciplinary approach than has been the case with standard lateral flow immunoassays. Input is required from a range of disciplines, including materials science, chemistry, biology, optics, software and hardware engineering, as well as process design, equipment design, and project management. For this reason, a more collaborative approach is required, and companies established with the purpose of fulfilling the many needs of developers in this complex area. Clear license for use, free of other patent infringement Careful application of lateral flow technologies in well-chosen market areas, coupled with robust, simple reading technologies, novel materials, the correct labels, modified device designs, and appropriate manufacturing strategies, will drive the acceptance of this technology in a vast array of application areas.

Chapter five

Summary and future work

5.1 Summary

Nanotechnology is a multidisciplinary field, as it combines the knowledge from different disciplines: chemistry, physics, and biology amongst others, The technology has excellent prospects for exploitation across the medical, pharmaceutical, biotechnology, engineering, manufacturing, telecommunications and information technology markets.

Nanotechnology offers important new tools expected to have a great impact on many areas in medical technology. It provides opportunities not only to improve materials and medical devices but also to create new smart devices and technologies where existing and more conventional technologies may be reaching their limits. It is expected to accelerate scientific as well as economic activities in medical research and development. Nanotechnology has the potential to make significant contributions to disease detection, diagnosis, therapy, and prevention.

. Biosensors could detect of diseases, within minutes, from blood samples or saliva or urine, early detection of illness and eliminate the need for maintaining large laboratories, transporting samples within facilities, and sending samples out for external analysis.

Biosynthesis of metal nanoparticles using plant derivatives is extremely studied in the last two decades. The plant metabolites induce the production of metallic nanoparticles in ecofriendly manner.

The ecofriendly synthesized GNP by fenugreek and black seeds extract studied using spectrometry and imaging methods to determine the formation of gold nanoparticles, its size and shapes .the Uv-vis spectrometer peaks between 534-536 for both depending on extract volume. XRD peaks are found to be broad indicating the formation of nanoparticles. FTIR measurements were carried out to identify the possible biomolecules present in fenugreek seed extract which are responsible for the reduction of gold NPs.

TEM image depicts the presence of spherical nanoparticles in black seed sample size range from 9nm to 29nm, for fenugreek seed GNPs presents in different shapes in same rang, when using 8ml extract the size increase.

5.2 Future work

- Develop immunochromatography assay and study it performance
- Synthesis nanoroads particles by fenugreek seeds and without using gum Arabic as stabilizer
- Study toxicity of GNPs syntheses by green method in test animals
- Develop non-enzymatic sensor for glucose

References

1-Filipponi, L. and Sutherland, D., 2013. Nanotechnologies: Principles, Applications, Implications and Hands-on Activities. *Publications Office of the European Union, Luxembourg*.

2- Alanazi, F.K., Radwan, A.A. and Alsarra, I.A., 2010. Biopharmaceutical applications of nanogold. *Saudi Pharmaceutical Journal*, *18*(4), pp.179-193..

3- Deb, S., Patra, H.K., Lahiri, P., Dasgupta, A.K., Chakrabarti, K. and Chaudhuri, U., 2011. Multistability in platelets and their response to gold nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*, *7*(4), pp.376-384.

4- Kuppusamy, P., Yusoff, M.M., Maniam, G.P. and Govindan, N., 2014. Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications–An updated report. *Saudi Pharmaceutical Journal*.

5- Li, X., Xu, H., Chen, Z.S. and Chen, G., 2011. Biosynthesis of nanoparticles by microorganisms and their applications. *Journal of Nanomaterials*, *2011*, p.8..

6- Tiwari, P.M., Vig, K., Dennis, V.A. and Singh, S.R., 2011. Functionalized gold nanoparticles and their biomedical applications. *Nanomaterials*, *1*(1), pp.31-63.

7- Zhu, Y.J. and Chen, F., 2014. Microwave-assisted preparation of inorganic nanostructures in liquid phase. *Chemical Reviews*, *114*(12), pp.6462-6555..

8- Sneha, K., Sathishkumar, M., Lee, S.Y., Bae, M.A. and Yun, Y.S., 2011. Biosynthesis of Au nanoparticles using cumin seed powder extract. *Journal of nanoscience and nanotechnology*, *11*(2), pp.1811-1814.

9- Fragoon, A., Li, J., Zhu, J. and Zhao, J., 2012. Biosynthesis of controllable size and shape gold nanoparticles by black seed (Nigella sativa) extract.*Journal of nanoscience and nanotechnology*, *12*(3), pp.2337-2345.

10-S. Aromal, S.A. and Philip, D., 2012. Green synthesis of gold nanoparticles using Trigonella foenum-graecum and its size-dependent catalytic activity.*Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *97*, pp.1-5.

11- Been Huang Chiang, 2013, Enzyme and Nanomaterial Based Optical and Electrochemical Biosensors for Plant Pesticides Detection.

12- Saha, A. and Datta, A.K., 2002. Gamma-rays Induced Reciprocal Translocation in Black Cumin (Nigella sativa L.). *Cytologia*, *67*(4), pp.389-396.

13-Spectronic, T., Basic UV-Vis Theory, Concepts and Applications< http://www. thermo. com/eThermo/CMA/PDFs. *Articles/articlesFile_12067. pdf>(13 Maret 2011)*. 14. Speakman, S.A., 2010. Basics of X-Ray Powder Diffraction. *Massachusetts-USA, 2011a. Disponível em:< http://prism. mit. edu/xray/Basics% 20of% 20X-Ray% 20Powder% 20Diffraction. pdf*.. (617) 253-6887

15- Stuart, B., 2005. *Infrared spectroscopy*. John Wiley & Sons, Inc...

16- Tyutyunnikov, D., 2010. *High resolution transmission electron microscopy investigations of FeP t and Au nanoparticles* (Doctoral dissertation, Dissertation, University Duisburg-Essen. Germany).

17- Bukhari, S.B., Bhanger, M.I. and Memon, S., 2008. Antioxidative activity of extracts from fenugreek seeds (Trigonella foenum-graecum). *Pakistan Journal of Analytical & Environmental Chemistry*, *9*(2).19- Faraday, M. The bakerian lecture: Experimental relations of gold (and other metals) to light. Philos. Trans. R. Soc. Lond. **1857**, 147, 145–181.

20- Tan, J., 2008. Analysis of Whole Blood Samples: Optimization of Sample Preparation for Rapid Assays.

21- Khan, A.K., Rashid, R., Murtaza, G. and Zahra, A., 2014. Gold nanoparticles: synthesis and applications in drug delivery. *Trop J Pharm Res*, *13*(7), pp.1169-1177.,

22- Conroy, P.J., Hearty, S., Leonard, P. and O'Kennedy, R.J., 2009, February. Antibody production, design and use for biosensor-based applications. In*Seminars in cell & developmental biology* (Vol. 20, No. 1, pp. 10-26). Academic Press.

23- Virekunnas, L., 2012. Colloidal Gold and Paramagnetic Labels in Lateral Flow Assay Detecting Ricin.

24- Raphael C. Wong, Harley Y. Tse, 2009, Lateral Flow Immunoassay, LLC

25- Singer, J.M. and Plotz, C.M., 1956. The latex fixation test: I. Application to the serologic diagnosis of rheumatoid arthritis. *The American journal of medicine*, *21*(6), pp.888-892.

26- Vaitukaitis, J.L., Braunstein, G.D. and Ross, G.T., 1972. A radioimmunoassay which specifically measures human chorionic gonadotropin in the presence of human luteinizing hormone. *Am J Obstet Gynecol*, *113*(6), pp.751-758.

27- Dykman, L.A. and Khlebtsov, N.G., 2011. Gold nanoparticles in biology and medicine: recent advances and prospects. *Acta Naturae (англоязычная версия)*, *3*(2 (9)).**.**

28- Csáki, A., Möller, R. and Fritzsche, W., 2002. Gold nanoparticles as novel label for DNA diagnostics. *Expert review of molecular diagnostics*, *2*(2), pp.187-193.

29- Xue, C., Arumugam, G., Palaniappan, K., Hackney, S.A., Liu, H. and Liu, J., 2005. Construction of conjugated molecular structures on gold nanoparticles via the Sonogashira coupling reactions. *Chem. Commun.*, (8), pp.1055-1057.

30- Parida, U.K. and Nayak, P., 2012. Biomedical Applications of Gold Nanoparticles: Opportunity and Challenges. *World Journal of Nano Science & Technology*, *1*(2), pp.10-25..

31- www.sigmaaldrich.com/materials-science/nanomaterials/ gold nanoparticles

32- Mittal, J., Batra, A., Singh, A. and Sharma, M.M., 2014. Phytofabrication of nanoparticles through plant as nanofactories. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, *5*(4), p.043002.

33- Yetisen, A.K., Akram, M.S. and Lowe, C.R., 2013. Paper-based microfluidic point-of-care diagnostic devices. *Lab on a Chip*, *13*(12), pp.2210-2251.

34- Lee, L.G., Nordman, E.S., Johnson, M.D. and Oldham, M.F., 2013. A low-cost, high-performance system for fluorescence lateral flow assays.*Biosensors*, *3*(4), pp.360-373.

35- JEM-2100 ELECTRON MICROSCOPE instruction IEM210-1 OCT2004-01110010 Printed in Japan.

36- Qian, S. *Biochemistry*, *322*(1), pp.89-98.

and Bau, H.H., 2003. A mathematical model of lateral flow bioreactions applied to sandwich assays. *Analytical.*