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Chiral Colorimetric Detection Of Amino Acids Based On L-Cysteine-Capped Silver Nanoparticles

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Dedications

This dissertation is dedicated to my most important people in my life:

To my dear parents for all their sacrifices, love, tenderness, support and prayers throughout my life. My dear father Aissa and my adorable mother Biri, you are the bed stone and the essential pillar in my journey.

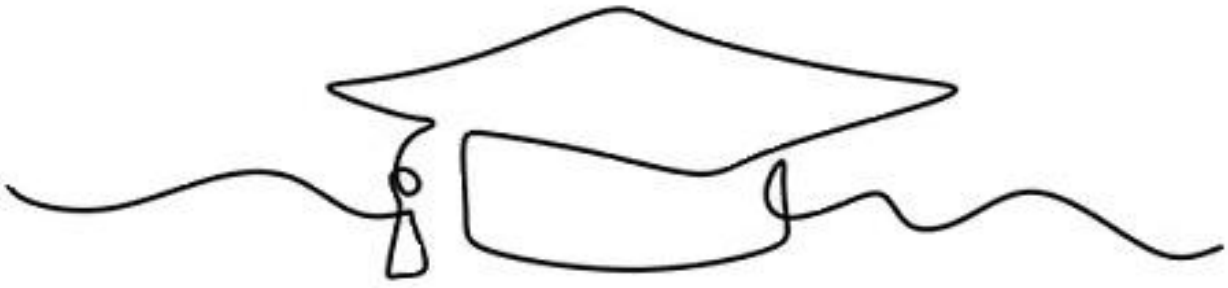
To my dearest sister Khadra and my dear brothers Ahmaida and Tahar for their support; May God protect them and give them luck and happiness.

To all my teachers during my years of study, you all supported me throughout this journey.

To all my friends and loved ones who have always encouraged me.

To all of you, I dedicate this work.

Ibtissam

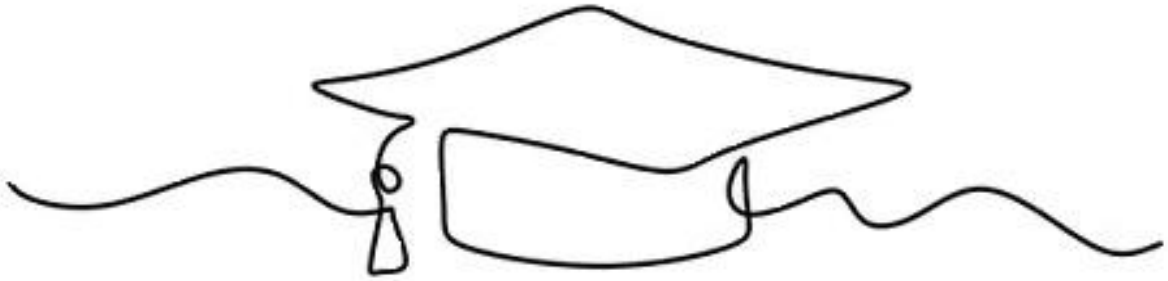


Dedications

To my dear father, may God have mercy on you, to my compassionate mother, to my brothers, my heroes, to my husband my everything , I own.....

To all my family members, I dedicate to you the fruit of my effort and the joy of my graduation and my humble achievement , My friend and companion, IBTISSAM I would like to thank you for all your efforts and your keen interest in our research. I wish you success and happiness in your professional and daily life.

Fatna



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List of abbreviations /Symbols

Ag: Silver metal

AgNPs: Silver nanoparticles

Ag+: Silver ion

Ag0: Neutral charge silver

°C: Celsius unit of temperature

DRX: X-ray Diffraction

FT-IR: Fourier Transform Infrared Spectroscopy

g/cm3: Grams per cubic centimeter (measuring of density)

HPLC: High Performance Liquid Chromatography

L-Cys: L-Cysteine

L-Lys: L-Lysine

MET: Transmission Electron Microscopy

MEB: Scanning Electron Microscopy

mg: Milligram

nm: Nanometer

pH: Hydrogen Potential

PVP: Poly Vinyl Pyrrolidone

SPR: Surface Plasmon Resonance

u: Unified atomic mass unit (atomic mass unit)

UV-vis: UV-Visible Spectroscopy.

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GENERAL INTRODUCTION

General introduction

Nanotechnologies and nanosciences are a field of research and technological development. In recent years, due to their use in various sciences such as energy, medicine, pharmaceutical industries, electronics and space industries etc., this technology deals with small structures and small sized materials ranging from a few nanometers to less than 100 nanometres[1]. Nanoparticles (NPs) have unique chemical, physical, biological, dielectric, electrical, thermal, optical , electronic, magnetic, mechanical , and photocatalytic properties that are significantly different from matter. These special and unique properties could be related to their small dimensions, large surfaces, shape, and size (1 to 100 nm)[2]. Nanoparticles are characterized by their unique magnetic, optical, electronic, and catalytic properties, which differ from solid particles or molecules by their size and shape, making their use in different applications very important[3].

Silver nanoparticles (AgNPs) are probably the most remarkable members of metal nanoparticles. They have a variety of applications in nanomedicine and, in fact, they represent perfectly suited tools not only due to their synthesis but also because of their great surface functionalization capacities, through the attachment of sulfur or amino molecules, biological molecules, and antibodies[4]. The interface properties of a silver surface functionalized with a self-assembled monolayer will be adjusted by the features on the external side of the monolayer. The synthesis, functionalization, and surface structure of silver nanoparticles protected by a monolayer have been intensively studied recently[5].

The synthesis of nanoparticles can be carried out by various methods, such as physical, chemical, and biological approaches. In general, physical and chemical methods are considered the best for obtaining uniform nanoparticles with long-term stability and environmental conservation, without harmful chemical waste, whether solid or gaseous. For this reason, we are conducting this work based on the chemical synthesis of silver nanoparticles, which is a more economical and efficient procedure. We have therefore adopted this method by reducing the silver ions (Ag+) in the solution ($AgNO_3$) using reducing agents like sodium borohydride or citrate[6].

In addition, the aggregation and dispersion of colloidal silver nanoparticles is one of the key issues related to their potential applications. Here particular attention was paid to controlling colloidal stability using sodium citrate.

In this context, the overall objective of this work is to study the possibility of silver nanoparticles synthesis by sodium citrate and their characterization by UV-Visible spectroscopies ,infrared FT-IR and transmission electron microscopy (TEM) . Reasonable chiral discrimination has been achieved using silver nanoparticles technique while improving the sensitivity of chiral recognition is essential and remains a difficult task. UV-Visible spectroscopy is expected to be a feasible method for improving the sensitivity of analysis discrimination.

We present in this research work, the synthesis of silver nanoparticles coated with the amino acid L-Cysteine can spectacularly distinguish L-Lysine. The detection sensitivity improved by almost 500 times using the silver nanoparticle method. In short, the method mentioned below makes the detection of chiral amino acid derivative much easier than conventional instrumental analyses.

In describing this work, it begins with a general introduction that provides an idea of the importance of the addressed theme while clearly explaining the objective.

This manuscript is divided into three chapters::

The first chapter summarizes the whole theoretical part, it is devoted to the generalities about nanoparticles, their methods of development as well as their properties, characterization and their applications in the various fields. We also present bibliographic syntheses of the deferential methods used for the preparation of AgNPs.

In the second chapter we present the experimental part in the first place, the method and experimental protocols used for the synthesis of silver nanoparticles. Then, a presentation of the different characterization techniques.

The third chapter is reserved for discussion of the results obtained and interpretations.

Finally, a conclusion resumes all the results obtained in this work as well as some recommendation for future studies.

CHAPTER I
BIBLIOGRAPHIC STUDY

I.1. Introduction

Nanoparticles attract attention in a range of scientific and technological disciplines. These nanoparticles, when examined individually, have distinct properties from their aggregate counterparts. This is due to their unique physical and chemical properties that differ from those in the raw state. The importance of its potential technological applications is equal to its usefulness as a basic model for the study of material growth.

I.2. Nanoparticles

1.2.1 Definition

Nanoparticles are small particles ranging from 1 to 100 nanometers in size, invisible to the human eye. They have unique physical and chemical properties compared to larger materials. The European Commission defines a nanoparticle as having at least half of the particles in the number distribution with a size below 100 nm. Nanoparticles have a large surface area to volume ratio, allowing them to possess unexpected optical, physical, and chemical properties due to quantum effects[7]. They can be classified into organic or inorganic, carbon-based, ceramic, semiconducting, or polymeric, and can be hard or soft depending on their application, such as industrial catalysis, environmental remediation, and composites [8].

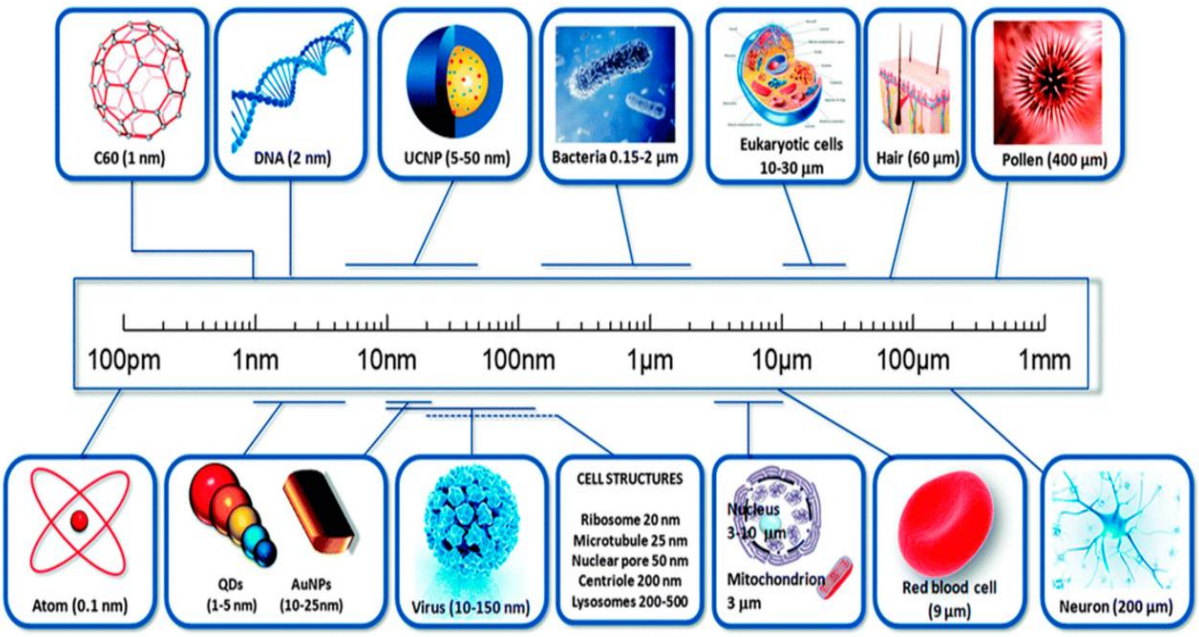


Figure I. 1: Basic comparison of nanomaterial sizes[9].

I.2.2. Nanoparticle Origin

Nanoparticles have a diverse origin, with some being naturally produced and others resulting from human activities. They can be classified based on their origin, composition, and morphology; there are three types according to their origin.

I.2.2.1. Nanoparticles of natural origin

Nanoparticles of natural origin are produced through various physical, chemical, and biological processes in different environmental compartments, such as (bio)chemical weathering of minerals, photo-oxidation, redox and precipitation reactions, (bio)mineralization, physical fragmentation, and gas-solid nucleation in the atmosphere. These natural nanoparticles are formed by chemical, photochemical, mechanical, thermal, and biological processes separately or in combination, including extraterrestrial processes like the production of cosmic dust. The mass of naturally occurring inorganic nanoparticles is estimated to be several thousand teragrams per year, significantly higher than the mass of engineered nanoparticles produced annually[10]. Natural nanoparticles play a crucial role in the Earth's biogeochemical system, moving through different compartments like the biosphere, lithosphere, atmosphere, and hydrosphere within the global biogeochemical cycle[11].

I.2.2.2. Non-intentional anthropogenic nanoparticles

Non-intentional anthropogenic nanoparticles are produced unintentionally due to human activities or processes, such as vehicular exhaust, combustion reactions, forest fires, and industrial emissions. They can also be found in insects, plants, and humans[12]. The concentration of these nanoparticles is high in areas with high urbanization, industrialization, and vehicular emissions, as well as in regions affected by extreme events. Most anthropogenic nanoparticles are made up of carbon, silicon, and metals[12]. These nanoparticles can significantly impact human health, particularly the respiratory system, causing respiratory and cardiovascular diseases and causing toxicological, hemotoxic, and tumorigenic effects.

I.2.2.3. Intentionally anthropogenic nanoparticles

Intentional anthropogenic nanoparticles are intentionally produced by humans for specific purposes, such as Nano medicine, electronics, catalysis, and environmental remediation. These nanoparticles are engineered to have specific properties, making them

suitable for targeted applications like drug delivery systems, sensors, and advanced materials. Researchers and scientists design and synthesize these particles to exhibit desired characteristics, such as size, shape, surface properties, and chemical composition, to meet specific industrial or scientific needs. These nanoparticles are designed to enhance performance, efficiency, and functionality in various sectors, including healthcare, energy, and environmental [13].

I.2.3. Classification of nanoparticles

Nanoparticles can be classified according to their size, morphology, physical and chemical properties.

I.2.3.1. Classification of nanoparticles by size

Depending on their dimensions, nanomaterials are classified into four different categories.

1. Zero-dimensional nanomaterials (0-D): this class has all three dimensions in the nanometric range, such as quantum dots, fullerenes.

2. Unidimensional (1-D) nanomaterials: this class has a dimension outside the nanometer scale, such as nanotubes, nano fibres, nano threads.

3. Two-dimensional (2-D) nanomaterials: this class has two dimensions outside the nanometer scale. like nano leaves, nano films

4. Three-dimensional (3-D) nanomaterials: in this class, materials are not confined to the nanometer scale in any dimension. This class contains powders, networks, etc [14].

I.2.3.2. Classification of nanoparticles by their composition

A.Organic nanoparticles: Organic nanoparticles, composed of organic compounds like proteins, carbohydrates, lipids, and polymers, are environmentally friendly, biodegradable, non-toxic, and economically viable. Ideal for drug delivery due to their hollow core and sensitivity to thermal and electromagnetic radiation, they are efficient in targeted drug delivery and cancer therapy[15].

- **polymeric nanoparticles :** These nanoparticles are made of polymers and are often used in drug delivery, sensor technology, and catalysis.

- **Lipid nanoparticles** : are generally spherical with a diameter between 10 and 100 mm. Their structure consists of a solid nucleus made of lipids and a matrix containing soluble lipophilic molecules. They have applications as drug and RNA release vectors in cancer therapy.

B. Inorganic nanoparticles: Inorganic nanoparticles, categorized into metal-based and metal oxide-based nanoparticles, are used in drug delivery due to their unique properties. These nanoparticles have better drug-loading capacity, stability, and tunable properties compared to organic nanoparticles. They can be incorporated into biomaterials to form composites, combining the advantages of organic biomaterials and inorganic nanoparticles. Examples of inorganic nanoparticles used for drug delivery include gold nanoparticles, silver nanoparticles, graphene-based nanomaterials, iron oxide, zinc oxide, hydroxyapatite, and cerium oxide nanoparticles[16].

- **Ceramic nanoparticles :** These nanoparticles are made of inorganic non-metallic solids such as oxides, carbides, carbonates, and phosphates. They are often used in photocatalysis, photodegradation of dyes, and biological imaging[16].
- **Metal nanoparticles :** are nanomaterials with a single element These nanoparticles are made of pure metals such as gold, silver, and copper. They are often used in electronics, catalysis, and biomedical applications[17].

C. Semiconductor nanoparticles : These nanoparticles have properties between metals and non-metals.They have been extensively researched for their potential in fields such as photocatalysis, nanoelectronics , nanophotonics, energy conversion, and biomedicine[18].

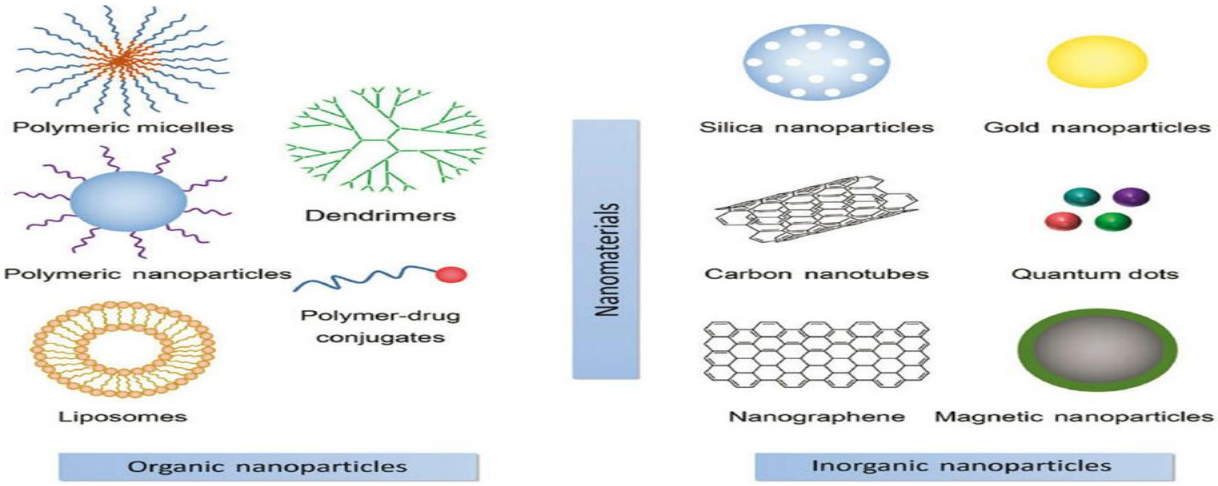


Figure I. 2: Representation of nanomaterials, including organic and inorganic nanoparticles[19]

D. Carbon-based nanoparticles: Carbon-based nanoparticles, composed of carbon, are a unique type of nanoparticle with excellent mechanical, electrical, thermal, optical, and chemical properties. These materials are suitable for various applications, including biomedical applications like imaging cells and tissues, drug delivery, solar energy scavenging, flexible electronics, molecular recognition, bio-imaging, sensing, tissue engineering, diagnostics, and biosensing. Carbon-based nanoparticles have high X-Ray absorption coefficient, ease of functionalization, and tunable properties, making them ideal for various applications. Their unique properties, such as high X-Ray absorption coefficient, ease of functionalization, and tunable properties, make them ideal for various applications[20].

I.2.4. Advantages and disadvantages of nanoparticles

Table I. 1: Some advantages and disadvantages of nanoparticles[21].

Advantages	Disadvantages
• Improve Rayleigh's broadcasting. • Improved Raman surface diffusion. • High plasma absorption. • Imaging of the biological system. • Determine chemical information on a metallic substrate on a nanometer scale [22]. • Increased specific surface area results in faster dissolution of the active agent in an aqueous environment, such as the human body [23].	• NPs' thermodynamic instability and high energy minima location can lead to degradation of quality, poor corrosion resistance, and difficulty in structure conservation. • NPs have been found to be toxic, carcinogenic, and irritating as they become transparent to the cellular dermis. • Exothermic combustion can cause an explosion, as fine metal particles act like powerful explosives. • Encapsulation is crucial for synthesizing nanoparticles, as maintaining their size in a solution form is challenging [22]. • NPs increase pollution [24].

I.3. Silver

I.3.1. Definition

Silver is a chemical element, a white lustrous metal valued for its decorative beauty and electrical conductivity. Silver is located in Group 11 (Ib) and Period 5 of the periodic table, between copper (Period 4) and gold (Period 6), and its physical and chemical properties are intermediate between those two metals. It is a malleable, ductile and precious metal that has been known since ancient times. Silver is widely distributed in nature, but the total amount is quite small when compared with other metals; the metal constitutes 0.05 part per million of Earth's crust. The atomic weight of silver is 107,880 and its atomic number is 47. Its basic electronic configuration is [Kr] 4d105s1. Silver can typically be found as a mixture of the isotopes 107Ag and 109Ag [25].

Figure I. 3:A silver nugget [25].

I.3.2. Physical and chemical properties of silver

• Silver is a transitional metal of silver-white color with a shiny metal gloss.

• Its atomic mass is 107.87 u.

• Its melting point is 961.78 °C, and its boiling point is 2,162 °C.

• Its density is 10.5 g/cm3.

• Silver is very ductile and very malleable, which means it can be easily stretched into thread and flattened into thin leaves.

• It is an excellent electrical and thermal conductor, about twice as good as copper.

• Silver is relatively inert chemically, but it can react with certain gases to form compounds such as silver oxide and silver sulfide.

• It has a valence of +1 and can form ionic compounds such as silver chloride (AgCl) and silver nitrate ($AgNO_3$).

• Silver is also known for its antimicrobial activity, making it a valuable material in medical and food applications [26].

I.4. Silver Nanoparticles

I.4.1 Definition

Silver nanoparticles are molecules with a size of 20–40 nm; they are composed of 80% silver atoms and 20% silver ions. These nanoparticles have specific properties that depend on their size and shape.

Scientific studies also show that catalytic activity is closely related to the size, structure, shape, and chemical environment of silver nanoparticles. Control of these parameters is often achieved by varying the methods of synthesis, reducing agents, and stabilizers [27].

I.4.2. Properties of silver nanoparticles

AgNPs have distinctive physico-chemical properties, including high electrical and thermal conductivity, improved Raman surface diffusion, chemical stability, catalytic activity, and nonlinear optical behavior.

I.4.2.1. Optical properties

Because of their size and form, silver nanoparticles (AgNPs) have special optical properties that are essential to their interaction with particular light wavelengths[28]. One important optical characteristic of AgNPs is surface plasmon resonance (SPR), which is the collective oscillation of free electrons in response to an electromagnetic field. They are useful in plasmonics and sensing applications because of their ability to interact strongly with particular light wavelengths[28][29]. AgNPs physical and chemical qualities are influenced by their size and form, which can vary from spherical to rod-shaped, triangular, or complicated. These characteristics include stability, solubility, and reactivity with biological

or environmental environments[29]. They are helpful in plasmonics and sensing as a result of their optical characteristics, which include considerable light absorption and scattering.

I.4.2.2. Thermal properties

AgNPs exhibit unique thermal properties, including high conductivity and constant specific heat capacity, making them useful in thermal management and composite materials, influenced by size, shape, aspect ratio, and temperature. A remarkable property of metal NPs is their low melting temperature due to thermodynamic size effect [30].

I.4.2.3. Catalytic Properties

From a chemical point of view, the decreased size of nanoparticles makes materials more reactive, making them promising candidates for catalysis applications. It has been experimentally demonstrated that metal nanoparticles have high catalytic activities for hydrogenation, carbonylation and hydroformylation . The catalytic properties of Ag NPs supported by silicon spheres were studied. Sharing AgNPs on silica spheres, effectively protect aggregation metal particles, thus avoiding deactivation and poisoning of catalysts during catalytic reaction[31].

I.4.3. Synthesis approaches

Nanoparticles synthesis methods are grouped into two major approaches.

The descending approach "Top-down" or commonly called (top-down method): this method consists in the gradual decrease of the size of an object to the mass state by means of appropriate reduction (high energy molding.....) until it reaches nanometric dimensions. In which nanoparticles are produced using physical techniques such as grinding or abrasion of a material[32].

The ascending approach "Bottom-up" or bottom-up approach: is to assemble atomic or molecular patterns to constitute nonmetric objects .Where nanoparticles are generated from “building blocks” of atoms or molecules, resulting in more complex assemblies[32].

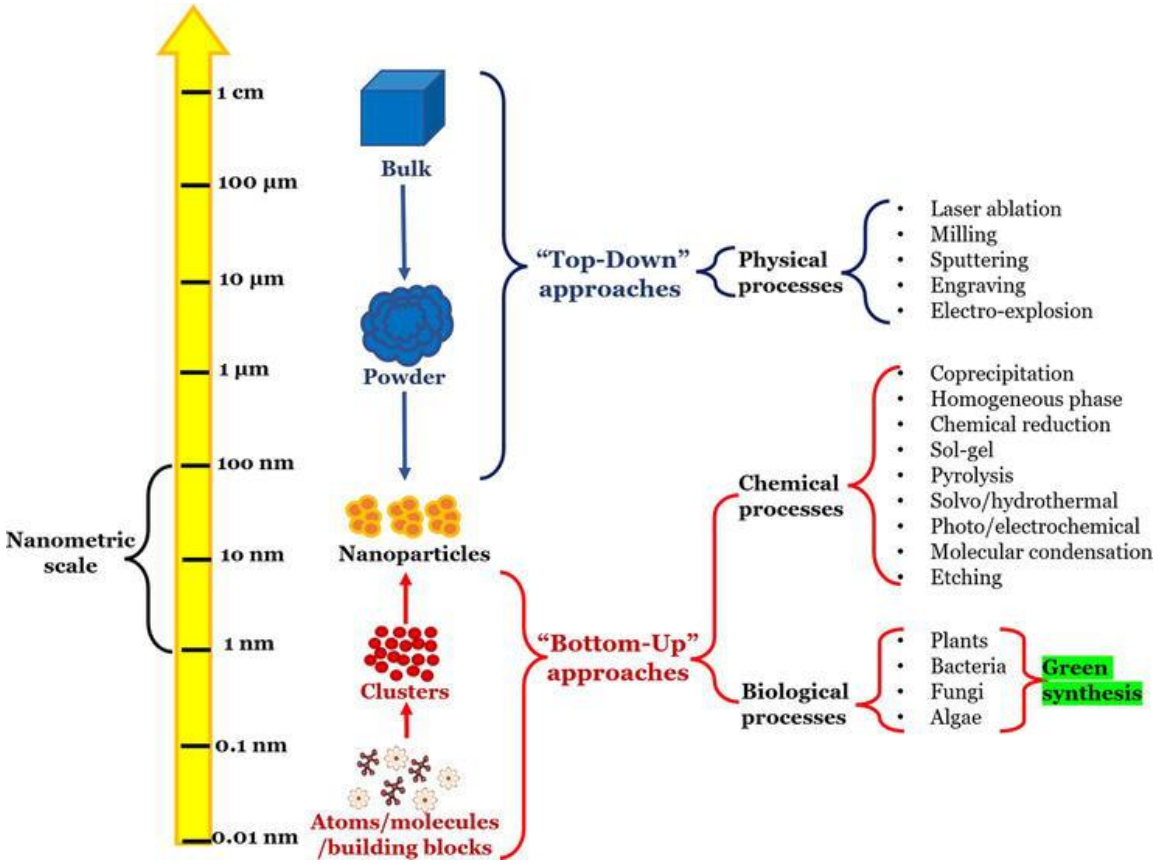


Figure I. 4: Nanometric scale and different approaches to nanoparticle synthesis[33].

1.4.4.Synthesis methods

Generally, the synthesis of nanoparticles (AgNPs) can be carried out using three different methods, including physical, chemical, and biological methods.

1.4.4.1.Physical Methods

The physical methods are generally fast, do not involve toxic chemicals and form a relatively narrow distribution of the size of the synthesized AgNPs. Several physical methods are possible for the synthesis of AgNPs. Examples include evaporation-condensation of a metal salt , ball grinding , discharge area , laser ablation. These methods can produce large quantities of AgNPs with high purity without the use of chemicals that release. However, Physical methods often face challenges like agglomeration due to the lack of capping agents, requiring complex equipment and higher power consumption, leading to higher operating costs[34].

1.4.4.2. Chemical Methods

Chemical synthesis methods include the Brust-Schiffrin synthesis and the Turkevich method. Chemical reduction of metal salts can be achieved using various chemical reductants like glucose, hydrazine, ascorbate, and sodium borohydride. The synthesis involves the reduction of silver ions (Ag+) in aqueous solutions, leading to the formation of colloidal AgNPs. Stabilizing agents are used to prevent agglomeration and oxidation of the nanoparticles[34].

- **Chemical ion reduction :**

The chemical reduction of silver ions (Ag^+) in aqueous solution is a common method for synthesizing silver nanoparticles (AgNPs). This bottom-up approach involves reducing silver salts like silver nitrate ($AgNO_3$) using various reducing agents in the presence of stabilizing agents[35].

Using a reducing agent such as glucose, ethanol, sodium citrate, or hydrazine hydrate, silver ions (Ag+) in a silver salt solution are reduced to silver atoms. After that, the silver atoms group together to form oligomeric clusters, which finally turn into colloidal silver particles.

To stop the nanoparticles from aggregating, stabilizing chemicals such as polyvinylpyrrolidone (PVP), linoleic acid, or citrate ions are added[35].

The following table groups together the different work using chemical reduction in solution to synthesize silver nanoparticles[36].

Table I. 2: Chemical reduction of silver in solution [36].

Solvant	Reducer	Stabilizing agent	Morphology and particle size
	Hydroxylamine Hydrochloride		Nanospheres
	Sodium Citrate	Sodium Citrate	Nanophiles or nanosphere 50-100 nm
	Tollens reactive		Nanospheres
	Raney Nickel		Ag's skeletal structure
Water	NaBH4	Dodekanethiol	Nanospheres 2-7nm
	Ethylene glycol	PVP	Nanofiles 30-60nm
	Ethylene glycol	PVP	Nanocubes

	Genamin T020 (non-ionic surfactant)	Multilamellar vesicles	Nanospheres3-9.5nm
	Phosphotungstate ion	Phosphotungstate ion	Nanoparticle network
	Dimethyl acetamide	PEG	Nanospheres
	DMF	PVP	Nanoprisms,Nanospheres
Organic	Acetonitrile	Tetrathiafulvalene	Dendritic Nanoparticles
	Ascorbic acid	Stem-shaped micelles	Nanotriangles
	Free radicals	AAO	Nanofiles

1.4.4.3.Biological Methods

Biological synthesis methods involve the use of plant extracts or microorganisms to reduce metal ions and synthesize AgNPs. These methods are considered green syntheses due to their high yield, solubility, and stability. Biological synthesis methods are attractive for their eco-friendliness and the ability to produce AgNPs with unique properties. The bio-reduction process typically involves mixing a metal solution with plant extracts at room temperature[34]. Each method has its advantages and considerations in terms of efficiency, cost, and environmental impact.

I.5. Characterization of AgNPs

Characterization plays a crucial role in identifying AgNPs based on their size, shape, morphology, structure, surface chemistry, surface load, dispersion and surface area. Different methods are used to characterize AgNPs , as mentioned below.

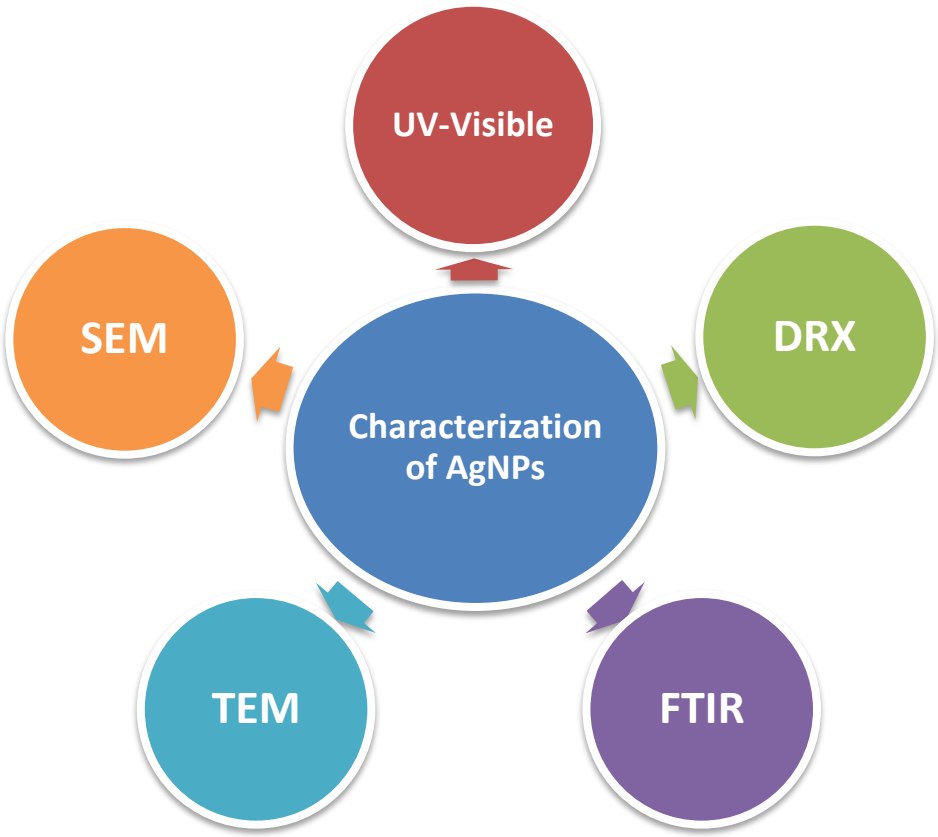


Figure I. 5: Silver Nanoparticles Characterizations

I.5.1. UV-Visible Spectroscopy

UV-vis spectroscopy is a very useful and reliable technique for the primary characterization of synthesized nanoparticles which is also used to monitor the synthesis and stability of AgNPs [35]. The absorption spectrum of AgNPs varies depending on the morphology, shape, size, and chemical environment of the synthesized nanoparticles. According to numerous studies, AgNPs has been demonstrated to generate absorption bands measuring approximately 200–800 nm in length. The spectrum of UV-visible waves has been used for the characterization of nanoparticles with a range of 2-100 nm[36] .

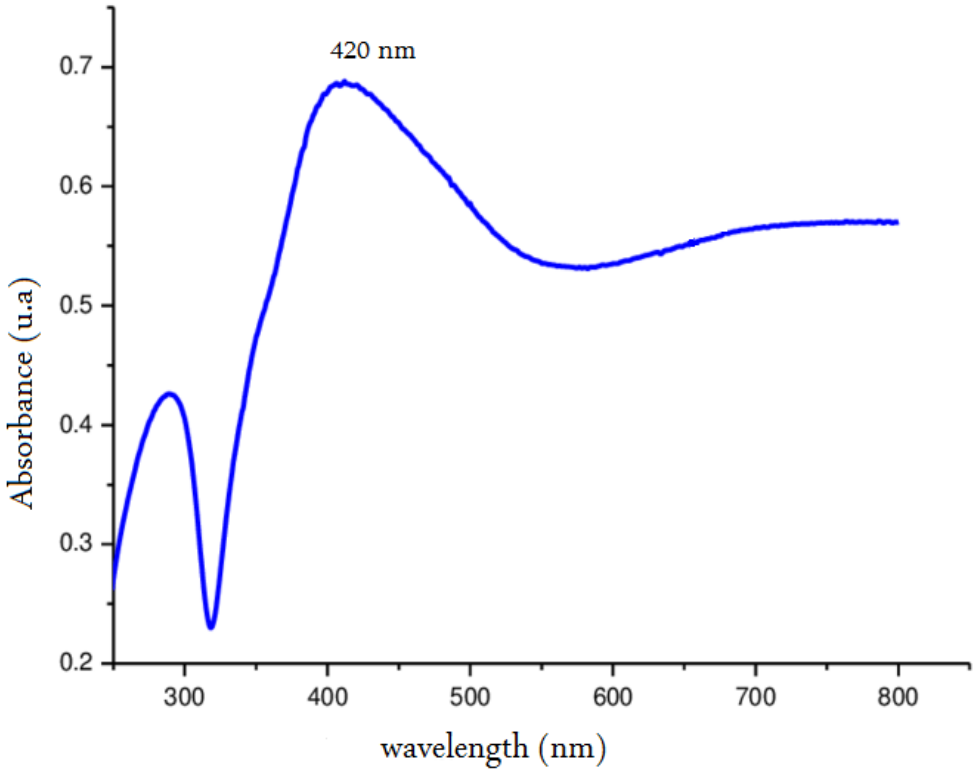


Figure I. 6:UV-visible absorption spectrum of an AgNPs solution [37].

I.5.2. Infrared spectroscopy to transform furry (FT-IR)

is used to study the surface chemistry of metallic nanoparticles and to find out whether biomolecules are involved in nanoparticle synthesis [35]. FT-IR is a non-invasive, appropriate, valuable, cost-effective, and simple technique to study the role of biomolecules in reducing $AgNO_3$ to silver [36].

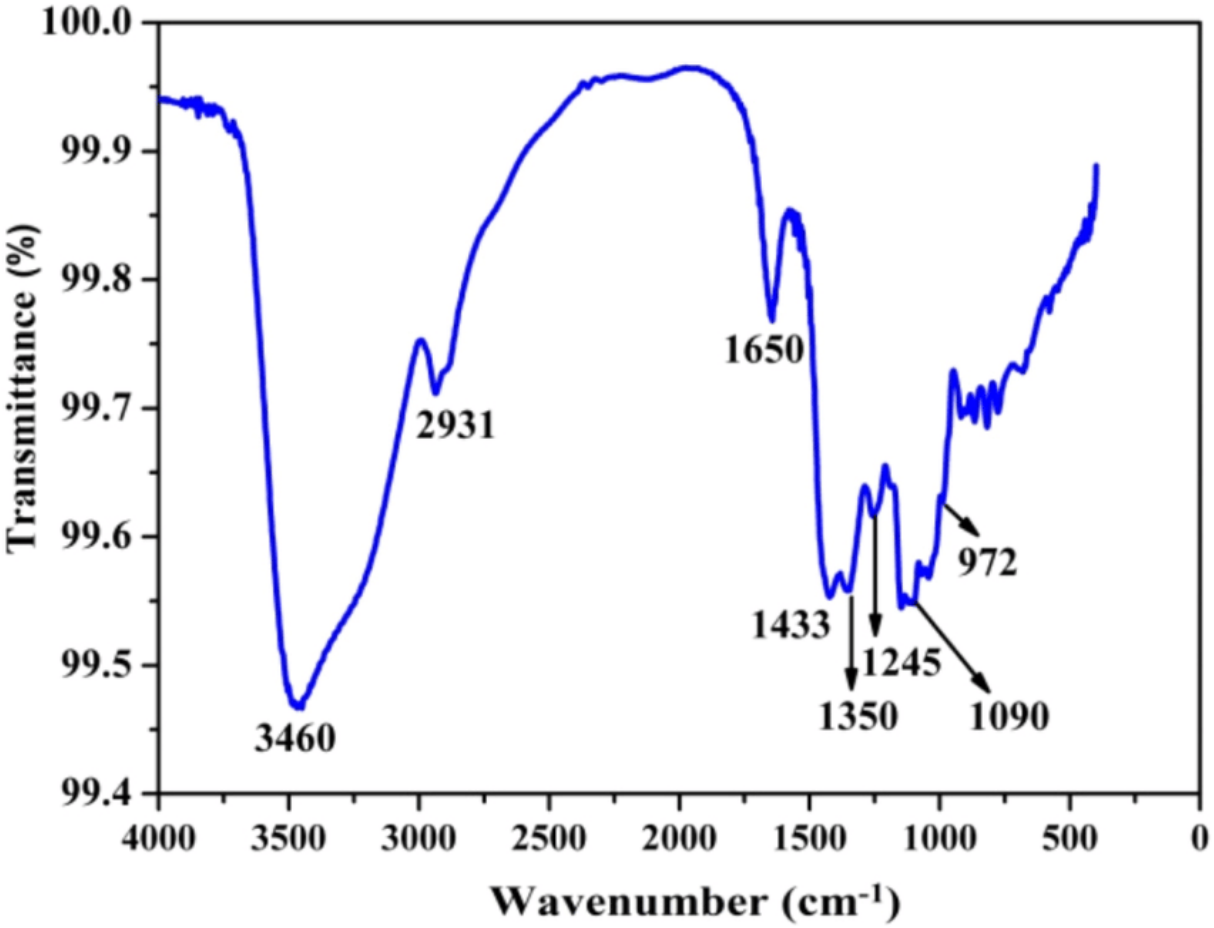


Figure I. 7: FTIR spectra of AgNPs [38].

I.5.3. The X-ray diffraction (DRX)

Phase identification and determination of the crystalline structure of NPs can be determined by X-ray diffraction (DRX) . With the help of DRX, the presence of AgNPs in the synthetic product can be confirmed. Confirmation is made by identifying the spectrum peaks of the DRX characteristic of the cubic crystalline structure with centered sides of metal silver [39].

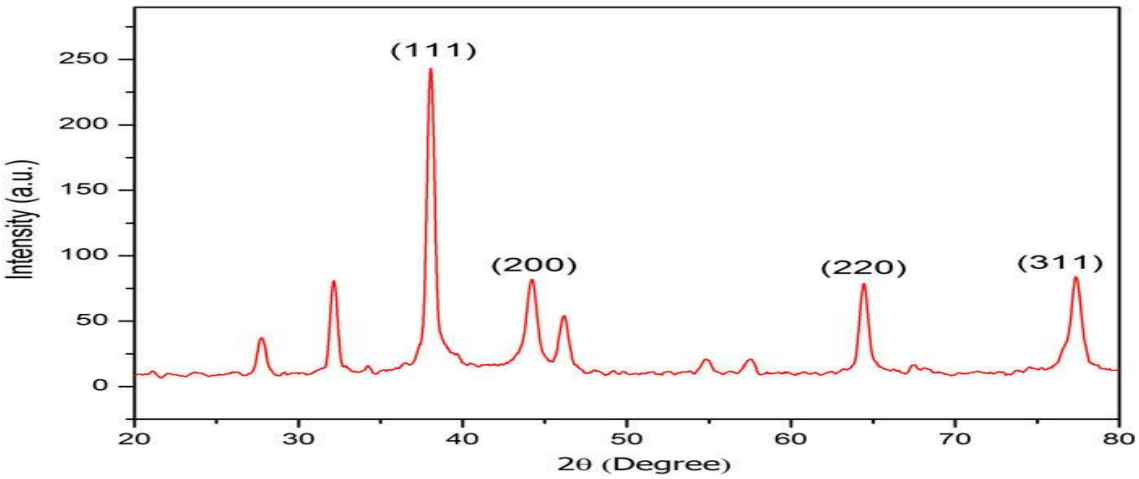


Figure I. 8:XRD spectrum of Ag-NPs at room temperature [40].

I.5.4. Transmission electron microscopy (TEM)

The (TEM) is an essential instrument for studying materials at the nanoscale. When used in conventional mode, it can determine the shape, size, polydispersity, and crystallinity of the synthesised nanoparticles. It is a highly high-performance technology with significantly higher resolution than the MEB, ranging from 0.1 to 1 nm. However, its manipulation is still delicate [39].

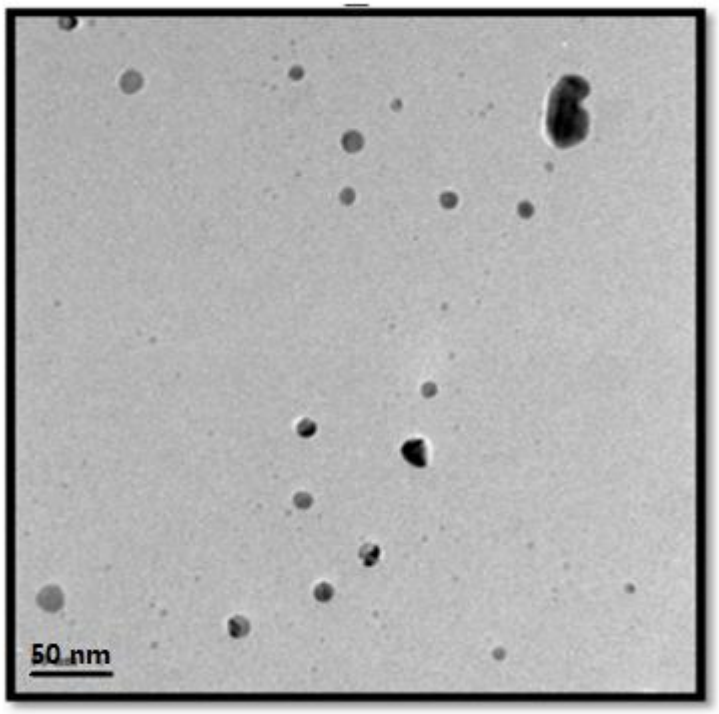


Figure I. 9:Transmission electron microscopy images of AgNPs [40].

I.5.5. Scanning electron microscopy (SEM)

It is a commonly used technique, its resolution remains quite good, and it varies between 1 and 7 nm. In addition to the observation of the microstructure, it allows for the identification of the local and even global chemical composition of the sample via the EDX analyser incorporated into the device.SEM uses a high-energy beam of electrons that is scanned on the surface of the sample of AgNPs then the observation of the retro-diffused electrons provides the specimen characteristics [39].

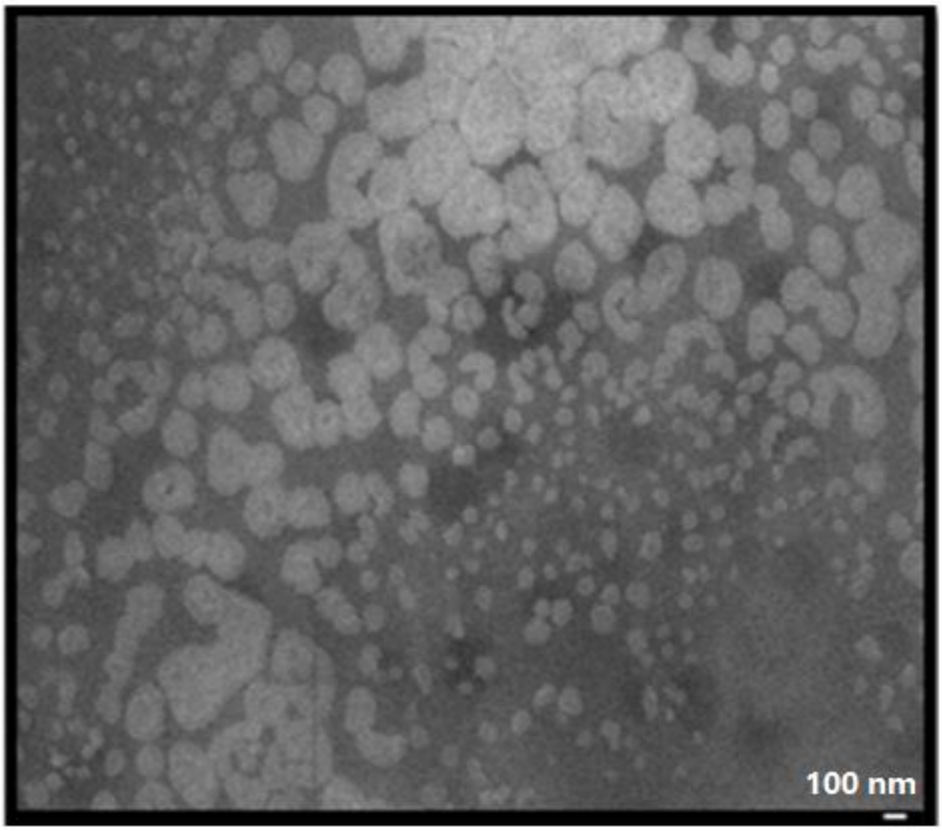


Figure I. 10: Scanning electron microscopy images of AgNPs [40].

I.6. Applications of silver nanoparticles

Silver nanoparticles have a wide range of applications across various industries due to their unique properties. These nanoparticles are extensively utilized in hygiene, medicine, and diverse sectors. They play a significant role in industries because of their remarkable characteristics like quantum confinement, high surface area, and small particle dimension [41].

The following table shows the applications of AgNPs in various areas:

Table I. 3: Applications of silver nanoparticles in different sectors [42].

Domain	Application of AgNPs
Biomedical Applications	• Antibacterial realization • Anti-fungal performance • Antiviral performance • Anti-inflammatory performance • Anti-cancer exploit
Applications textiles	• UV-blocking textiles • Textiles and medical devices
Food industries	• Nanotechnology and food packaging • Food processing
Environmental treatment	• Air Disinfection • Water Disinfecting ○ Drinking Water . ○ Groundwater and biological wastewater.
Pharmacological applications	• Antimicrobial Activity • Larvicidal Activities • Wound Healing Property
Applications conductrices	• LCD Displays • LED High Intensity • Touch Screens [
Catalyse	• Fuel cell catalyst • Fuel additive catalyst • Hydrogen production

I.7. Amino acids

I.7.1. Definition

Amino Acids are the organic compounds that combine to form proteins, hence they are referred to as the building components of proteins. These biomolecules are involved in several biological and chemical functions in the human body and are the necessary ingredients for the growth and development of human beings. There are about 300 amino acids that occur in nature. Altogether, there are twenty amino acids, which are involved in the construction of proteins [43].

Amino acids, with their general formula contain unique amino functional groups and a side chain containing an R-group. These R-groups differentiate amino acids and are responsible for their distinct properties, making them a valuable tool in various biological processes [44].

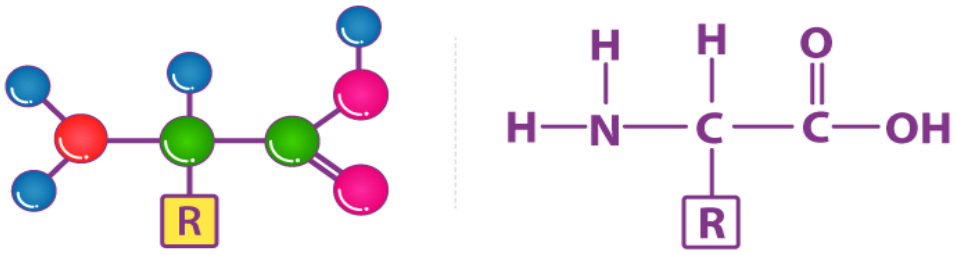


Figure I. 11: The general structure of Amino acids [44].

I.7.2. Physical-chemical properties of amino acids

A. Physical properties

- Amino acids are colorless crystalline substances.
- Most amino acids are insipid but some are sweet. (e.g. glycine, alanine) and some are bitter (Ex. Arginine) .
- Amino acids have a high melting point (200-300)^0 C due to the ionic property.
- The solubility of amino acids depends on the polarity, the isoelectric point, the nature of the solvent (pH) and the temperature.
- Amino acids are soluble in water and ethanol (i.e. a polar solvent) and insoluble in a non-polar solvent such as benzene, ether, etc.
- Amino acids are insoluble at the isoelectric point.
- Solubility depends on the pH of the solvent and temperature. For example ;Tyrosine is soluble in hot water .

B. Chemical properties

- The general formula for an amino acid is R-CH(NH_2)-COOH, where R represents the side chain that is unique to each amino acid and differentiates them.
- Amino acids are amphoteric, meaning they can act as both acids and bases, with the amino group being basic and the carboxyl group being acidic.

- The isoelectric point (pI) is the pH at which an amino acid has a net zero charge, with an equal number of positive and negative charges.
- All amino acids except glycine are chiral molecules, existing in two enantiomeric forms (L and D), but the L-enantiomers are the ones found in proteins.
- The side chain (R group) of an amino acid determines its unique chemical properties, such as polarity, charge, and reactivity.
- Amino acids can undergo various chemical reactions, such as decarboxylation, transamination, and deamination, which are important in metabolism and biosynthesis.

I.7.3. Applications of amino acids for modified surface of NPs

Amino acids are used to modify the surface of nanoparticles (NPs) in various applications. For example, L-Cysteine, an amino acid, was used to functionalize silver nanoparticles, which allowed specific structures to be created and used for the detection of amino acids. Amino acids, due to their functional groupings, can bind to the surface of nanoparticles and alter their physical-chemical properties. This surface modification can be crucial for improving the stability, reactivity, or interactions of nanoparticles in various fields such as biology, medicine, or catalysis [45].

Amino acids are essential in functionalizing silver nanoparticles (AgNPs), enhancing their biological activities, catalytic and oxygen sensing applications, and antimicrobial properties. FTIR spectroscopy can analyze the transformation of functional groups in AgNPs, affecting their cytotoxicity. The transformation of citrate-coated AgNPs in amino acids is crucial for human body fate [46].

It has also demonstrated that nucleotide-coated silver nanoparticles can be used as an ultra-high-efficiency enantio separation and detection platform for cysteine D- and L- [47].

I.8. Colorimetric detection of nanoparticles

I.8.1. Definition : The colorimetric detection of nanoparticles is a method of analysis that is based on the chemical reaction of nano-particles with reagents, this method allows to detect the presence of Nanoparticles in a sample by measuring the intensity of the coloring produced by the Chemical Reaction [48].

I.8.2 Applications of colorimetric chiral detection

Colorimetric chiral detection has various applications in different fields, particularly in the detection of biomolecules. The use of colorimetric sensors for chemical and biological sensing applications has been significant in recent years. These sensors have been employed for detecting chemical compounds, organic pollutants, heavy metal ions, and biomolecules[49]. Specifically, in the context of chiral detection, a study focused on the rapid, enantioselective, and colorimetric detection of D-arginine showcased the potential of incorporating multiple reaction sites on a chiral fluorescent probe to achieve specific responses to D-arginine among various chiral amino acids[50]. Additionally, the role of silver nanoparticles in colorimetric detection of biomolecules has been explored, highlighting the importance of Ag nanoparticles in detecting biomolecules like dopamine, proteins, and DNA with high sensitivity and selectivity, making them valuable tools for colorimetric and optical detection of biomolecules[51]. These applications demonstrate the versatility and significance of colorimetric chiral detection in various scientific and practical contexts.

I.9.Conclusion

The nanometric field is large. The first chapter discussed the various methods for the production and characterization of silver nanoparticles. Where we are, we addressed the origin and forms of nanoparticles, in addition to the utilization of silver in our work to synthesize silver nanoparticles, as well as the advantages and disadvantages of these particles,in particular the various applications of these nanoparticles.

CHAPTER II
EXPERIMENTAL SECTION

II.1.Introduction

In this chapter, we will describe the methodology used for the synthesis of silver nanoparticles and present the different operational parameters to optimize the synthesis. The set of descriptions of materials, products, methods, and analysis techniques used in the synthesis and characterization of nanoparticles is also detailed in this part.

II.2 Chemical method

Among existing methods, chemical methods have been the most widely used for the production of Ag NPs. The chemical reduction of metal ions is the most universal and easiest way to prepare metal nanoparticles. The chemical transformation of silver ions into silver nanostructures can occur using a photochemical method [1,2] wet chemical synthesis with. or without a model, using liquid crystals, polymer plates, and methodologies based on solutions such as aspartate reduction [1], and the reduction mediated by starch, etc. Generally, the chemical synthesis process of NP of Ag in solution uses the following three main components: (i) metal precursors (for the formation of AgNPs): $AgNO_3$, $AgClO_4$, AgCL, (PPh3), $3AgNO_3$, CF_3COOAg, (ii) reducing agents, and (iii) stabilizing agents or hatchers. Some of the representative reducing agents are: NaBH4, glucose, N, N-dimethyformamide, N_2H_4, sodium citrate, polyols (such as ethylene glycol, diethylen glycol, or a mixture of these), formaldehyde, etc…[3,4].

It is known that different reductors have varying levels of reducibility, which can play a crucial role in determining the ultimate shape of nanostructures. Furthermore, these reductors promote the growth of nanocrystals across their various surfaces.

II.2.1 Chemical Synthesis of Silver Nanoparticles

II.2.1.1 Method of Silver Nanoparticles Synthesis

For the preparation of silver nanoparticles we have chosen the synthesis of nanoparticle in liquid phase and at room temperature. The elements used are:

- Precursor which is the reactive.
- A reducing agent.
- A stabilizer.

The control of the growth rate and size of nanoparticles is done by controlling different parameters of the chemical reaction including the pH of the medium and the concentration of

the precursors. Searching for optimal conditions can lead to the formation of nanoparticles of the desired size.

The chemical production of silver nanoparticles begins with the reduction of metal precursors in solution, followed by precise procedures that determine nucleation and growth factors such as temperature, concentration, and ligand length. These parameters are critical in influencing the size and characteristics of produced nanoparticles.

II.3 Factors influencing the development reaction of Ag NPs

The synthesis reaction of AgNPs is very sensitive and depends on some important operational parameters. These are factors that influence the synthesis of NPs, regardless of the technique used. In this study, we evaluated several important experimental factors, including reaction temperature, pH, solution concentration of $AgNO_3$, reaction time, agitation, light, and ventilation.

A. Temperature :

The reaction temperature can influence the kinetics of nanoparticles' reduction and growth; higher temperatures can accelerate the reaction, while lower temperatures may promote the formation of smaller nanoparticle sizes [5].

B. The duration of the reaction:

May also influence the size and morphology of the silver nanoparticles formed, a longer reaction time may allow for further growth of the nanoparticle [6].

C. Stabilizers:

The addition of stabilizers, such as surface agents or polymers, may help to stabilize nanoparticles and prevent their aggregation or precipitation[7].

D. Agitation:

The degree of agitation of the reactive solution can affect the distribution of the sizes and shape of the formed nanoparticles, vigorous agitation can promote a more homogeneous dispersion of the nanoparticle[6].

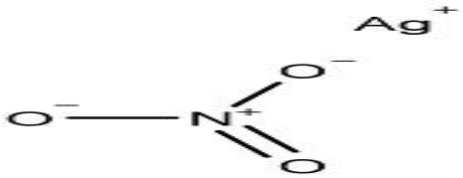
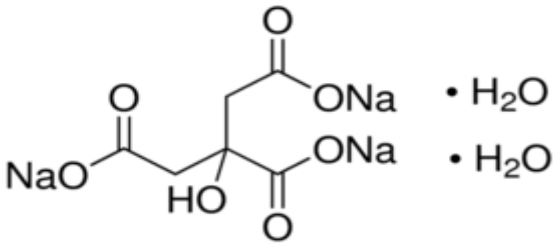
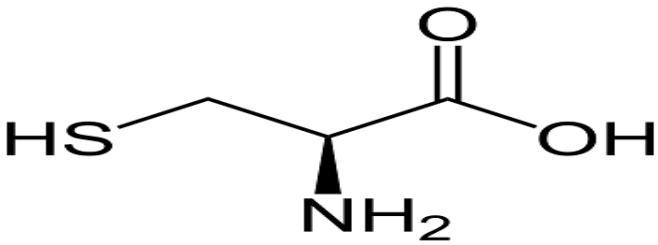
II.4 Materials and chemicals used

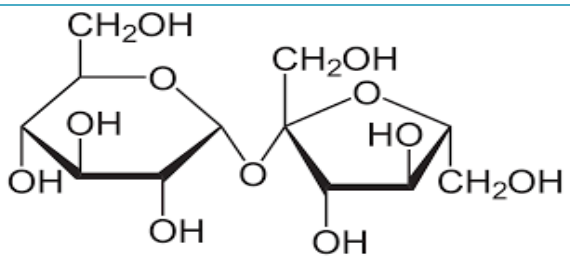
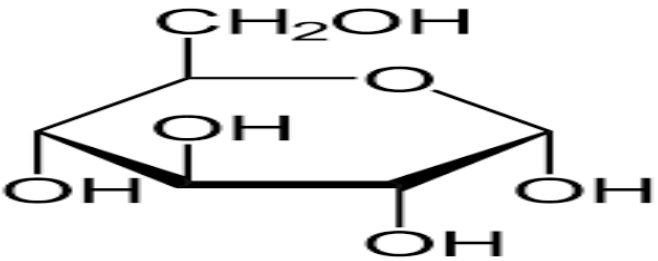
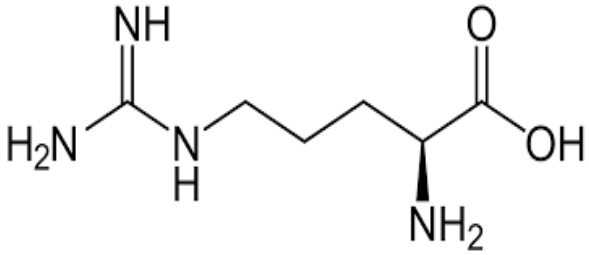
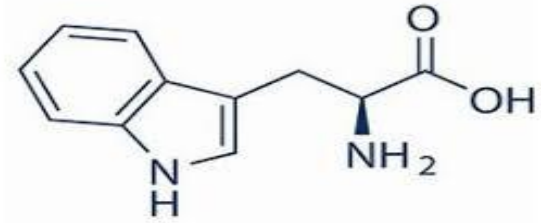
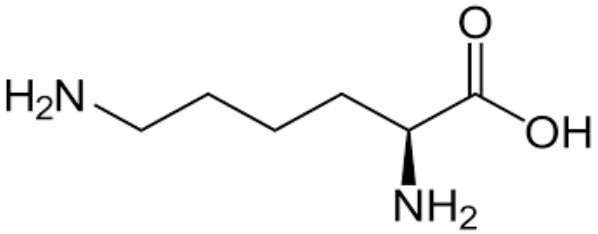
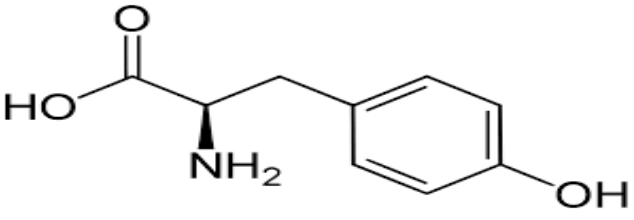
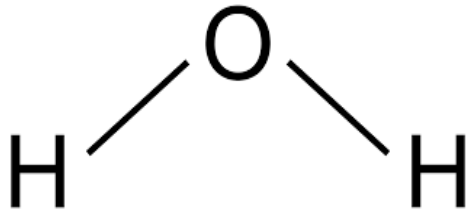
II.4.1 Materials used : Materials used in this work are :

- 250 ml balloon
- Refrigerant
- 50 ml graduated test tube
- 10 ml and 5 ml pipettes
- Wash bottle
- Spatula and brush
- 2 heating plates and magnetic stirrer
- 2 magnetic bars
- Test tube
- Precision Balance

II.4.2 Chemicals used:

Table II. 1:Chemicals used.

Products	Formula	M (g/Mole)	Structure
Silver Nitrate	$AgNO_3$	169,87	
Trisodium Citrate Diydrate	$C_6H_5O_7Na_3.2H_2O$	257,12	
L-Cysteine	$C_3H_7NO_2S$	121,16	

D-Sucrose	$C_{12}H_{22}O_{11}$	342,3	
D-Glucose	$C_6H_{12}O_6$	180,16	
L-Arginine	$C_6H_{14}N_4O_2$	174,2	
L-Tryptophane	$C_{11}H_{12}N_2O_2$	204,23	
L-Lysine	$C_6H_{14}N_2O_2$	146,19	
L-Tyrosine	$C_9H_{11}NO_3$	181,19	
Distilled water	H_2O	18	

II.5 Experimental protocol

Precursor products include $AgNO_3$ silver nitrate as an Ag ion source and $C_6H_5O_7Na_3.2H_2O$ Trisodium citrate di hydrate +as a reducing agent.

II.5.1 Development of silver nanoparticles

Citrate reduction synthesis is the first method to obtain a colloidal solution of silver by reducing silver nitrate with citrate into an aqueous solution [8]. This synthesis was developed by Lee and Meisel in 1982 and remains a classic approach to quickly and easily generating silver colloids. In this synthetic, a liquid solution of sodium citrate is rapidly added to a solution of $AgNO_3$ brought to a boil under vigorous agitation, it is reacted for an hour after a few minutes, the color of the solution passes from light yellow to red wine, then the reactionary medium is cooled, and the citrate ions act both as a reducing and stabilizing agent in this process [9].

The NPs generated using this approach are thermally stable and range in size from 1 to 20 nm.

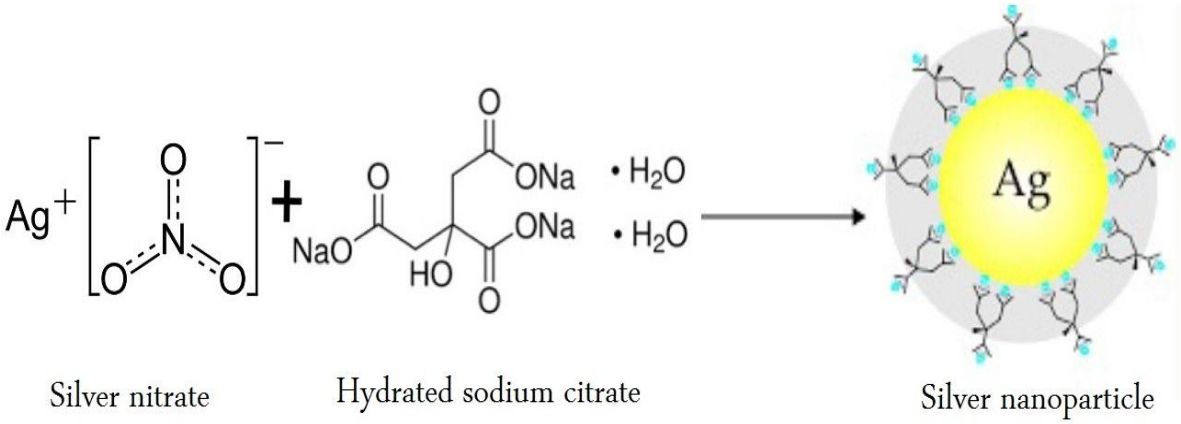


Figure II. 1 : Synthesis of Ag NPs by the citrate pathway .

The reaction mechanism can be represented as the following:

$$4Ag^{+} + C_6H_5O_7Na_3 + 2\,H_2O \rightarrow 4Ag^{0} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2\uparrow$$

II.5.2 Synthesis of silver nanoparticles

We dissolve **115 mg** of AgNO3 in **200 ml** of distilled water and pour it into a **250 ml** round-bottom flask (aluminium coating) equipped with a water condenser. We then bring the reflux mixture to a temperature of **70°C**. Once refluxing is achieved, we quickly add a solution containing **266 mg** of sodium citrate dissolved in **10 ml** of deionized water. The reaction is allowed to proceed for **45 minutes** under moderate stirring. During this time, the solution turns yellow-gold, indicating the formation of silver nanoparticles.

The formation of silver nanoparticles was further confirmed using UV-Vis spectroscopy.

Note

- The colloidal silver solutions were prepared, cooled to room temperature, and stored in carefully washed glass vials.
- It is important not to expose the solution to light after cooling.
- Cover the vessel to protect it from light during preparation.
- Store the solution at a temperature of 2–8 °C.
- Before beginning nanoparticle production, thorough cleaning of all glassware is necessary. It is recommended to clean all surfaces with aqua regia (a mixture of concentrated solutions of 70% nitric acid (HNO_3) and 37% hydrochloric acid (HCl)), while wearing gloves. Subsequently, rinse the glassware thoroughly with distilled water. Finally, dry the equipment on the laboratory oven.

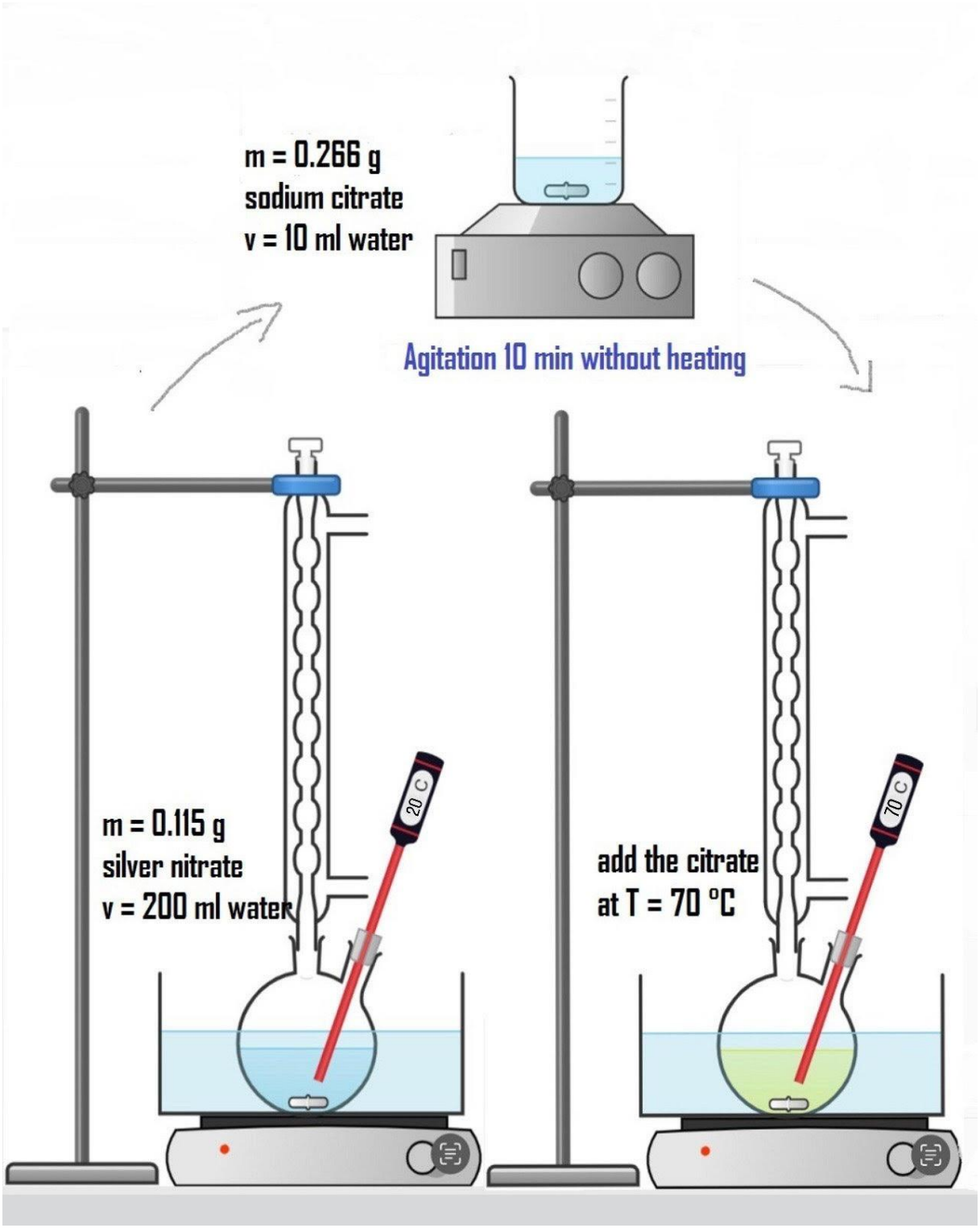


Figure II. 2: Scheme of synthesis of silver nanoparticles.

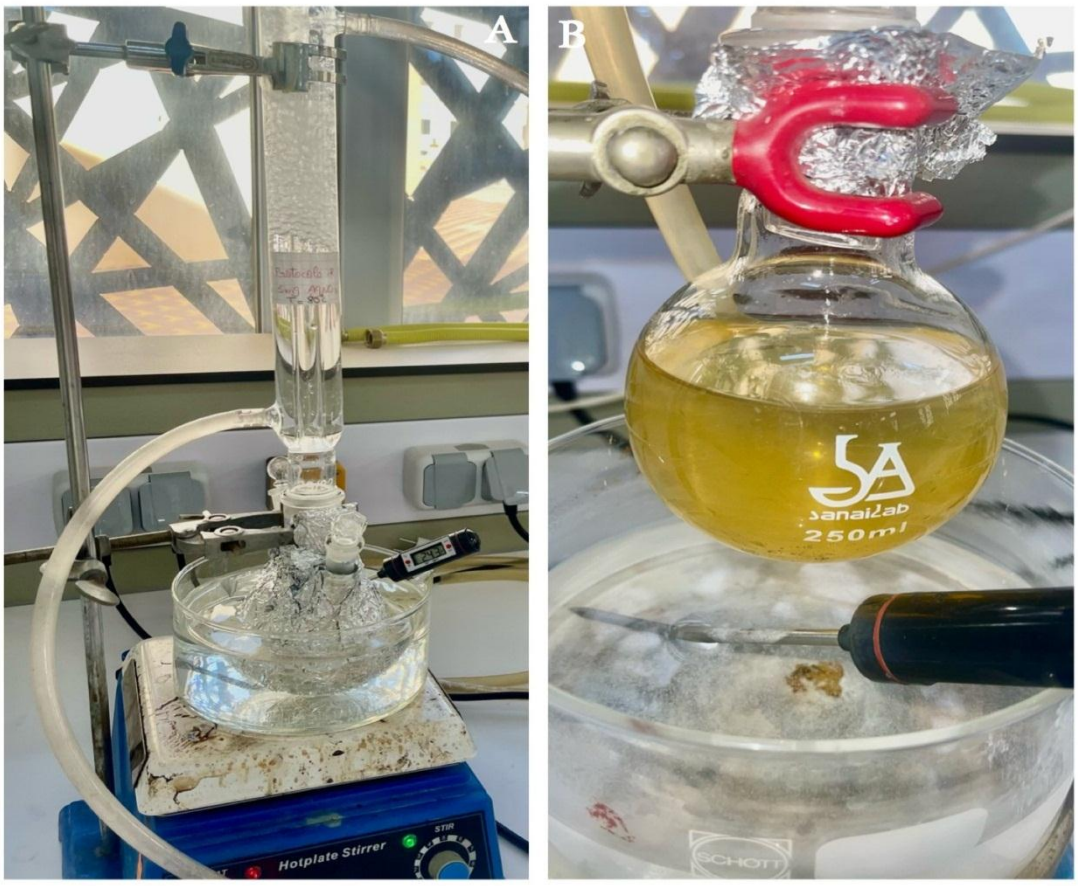


Figure II. 3:(A) AgNPs preparation assembly , (B) AgNPs solution.

II.5.3 Protocols of (L-Cysteine Ag NPs preparation)

After the silver nanoparticles have been synthesized, **6 mg** (**10^{-2} M**) of L-cysteine dissolved in **55 ml** of distilled water in a **100 ml** vial are prepared.

Take **5 ml** of this solution and add **10 ml** of the colloidal solution of AgNPs obtained with vigorous stirring for **2 h**.

In this research, we also used UV-Vis and Infrared spectroscopy to compare the colloidal solutions of modified AgNPs .

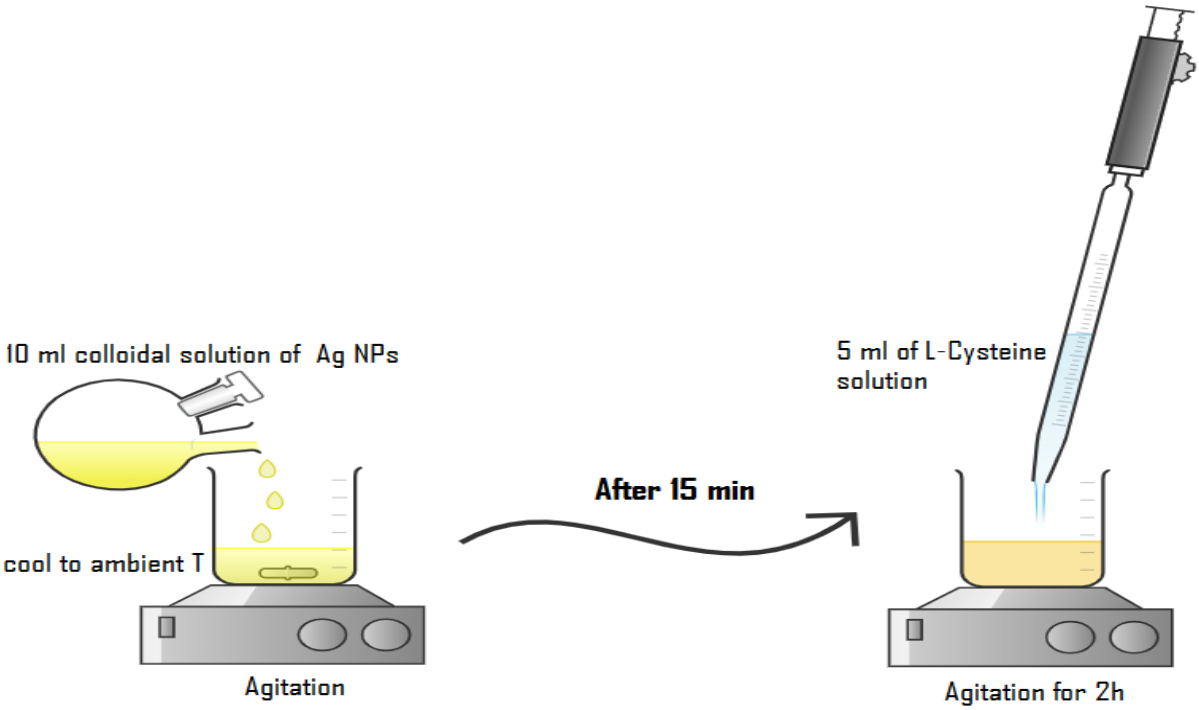


Figure II. 4:The scheme presents the protocol for the preparation of L-cysteine AgNPs .

II.5.4 Selective detection (color-metric tests)

In this part , we prepare a set of solutions using the compounds (amino acids and sugars) listed in Table II.2, where we take a concentration of $\mathbf{10^{-2}}$ **M** and dissolve it in **5 ml** of distilled water to obtain the solutions through which we perform selective detection.

Table II. 2: Sugar and acid mass used for colorimetric detection.

Product	Mass (mg)
D-Sucrose	17,11
D-Glucose	9
L-Arginine	8,71
L-Tryptophane	10,21
L-Lysine	7,31
L-Tyrosine	9,05

Small vials contain **0.5 ml** of different solutions.Then we pour **1.5 ml** of silver nanoparticle solution (AgNPs) into each vial, and after 5 minutes, the solutions are tested.

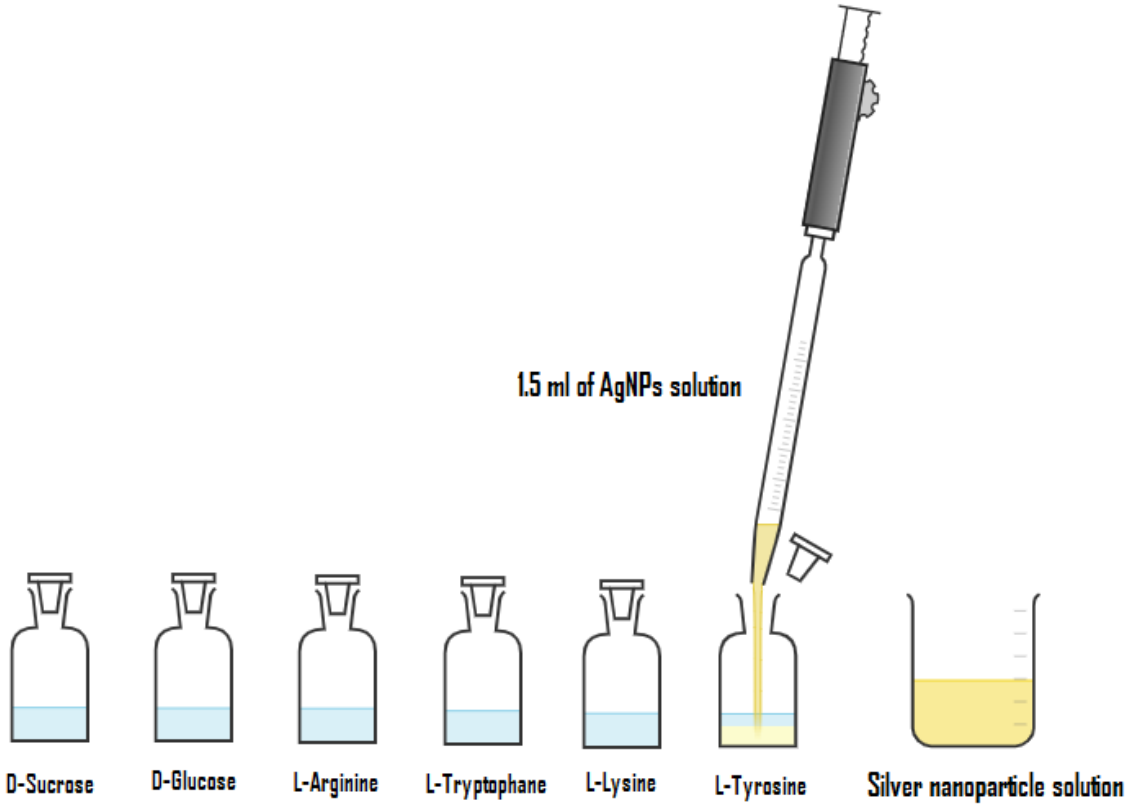


Figure II. 5:The scheme presents the color-metric tests.

II.6 Method of characterization of silver nanoparticles

II.6.1 UV-Visible Spectroscopy

UV-visible spectrophotometry is a quantitative method that measures the absorption of a substance in a clear solution using monochromatic light, thus allowing for determining the concentration of a substance in a non-destructive manner. This method applies to many dosages and can also be used to determine the precise concentrations of an absorbing compound using its molar extinction coefficient.

Absorption spectrometry is based on the absorption of radiation by molecules in the fields of ultraviolet and visible (190-800 nm). Silver metallic nanoparticles, gold, and copper have special optical properties due to the dielectric confinement effect.

We can consider UV-VIS radiation as an electromagnetic wave that carries an energy E linked to its frequency v by the ratio:

$$E = h.v = h.c / \lambda$$

With h Planck constant (h = 6.63 · 10^{-34} J · s), c speed of light in the medium where the wave spreads (in the vacuum), λ wavelength of radiation, usually expressed in nanometers (nm) [11].

UV Spectroscopy used model visible: Optizen2120UV, Nominal voltage: free voltage, Current assessed: 1A, Serial Numbers: 2U0101-1408029-05, Company: Ltd / Made in Korea. The device is connected to a microcomputer. The UV-Probe software allows the recording and visualization of the spectrum of the analysed samples.

Figure II. 6: Image of an ultraviolet spectrophotometer.

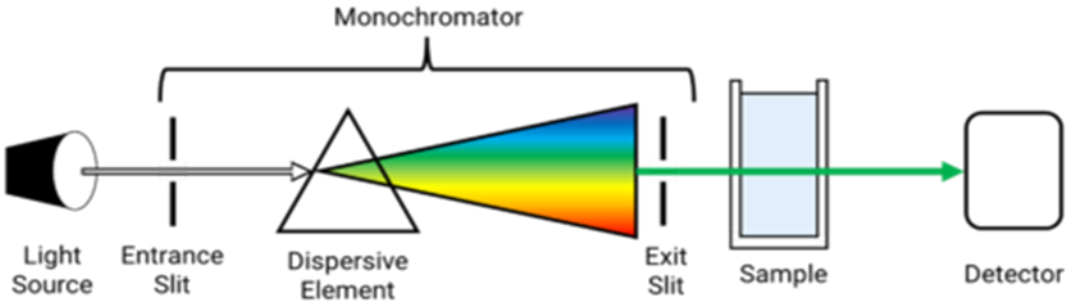


Figure II. 7: A basic block diagram of the elements in a single beam UV-Visible spectrometer[12].

II.6.2 Fourier transformed infrared spectroscopy (FTIR)

Infrared spectroscopy is used to confirm the interaction between silver and bioreceptors, it is a technique that allows the analysis of the composition of molecules by measuring the frequencies of the molecular vibration corresponding to the infrared field of electromagnetic radiation. Molecules can absorb the electromagnetic wave whenever the

frequency of the wave corresponds to one of their vibration frequencies, thereby identifying the composition of the molecule studied. This technique allows very precise measurements at low quantities of matter as well as the analysis of the composition and molecular structure of materials by detecting the vibrations characteristic of chemical bonds. Therefore, FTIR is a suitable, valuable, non-invasive, cost-effective, and simple technique to identify the role of biological molecules in the reduction of silver nitrate to silver [12].

We used a Fourier Transformed Infrared Spectrometer (FTIR) model FT/IR-4200 available at the Research Laboratory. Serial Numbers: C083761018, Power: 170 VA, Company: Jasco corporation 192-8537 / Made in JAPON. It is controlled by a micro-computer, the spectrum is recorded in the range 4000 to 400 cm^{-1} at room temperature.

Figure II. 8: Image of an infrared spectrophotometer (IVILBER).

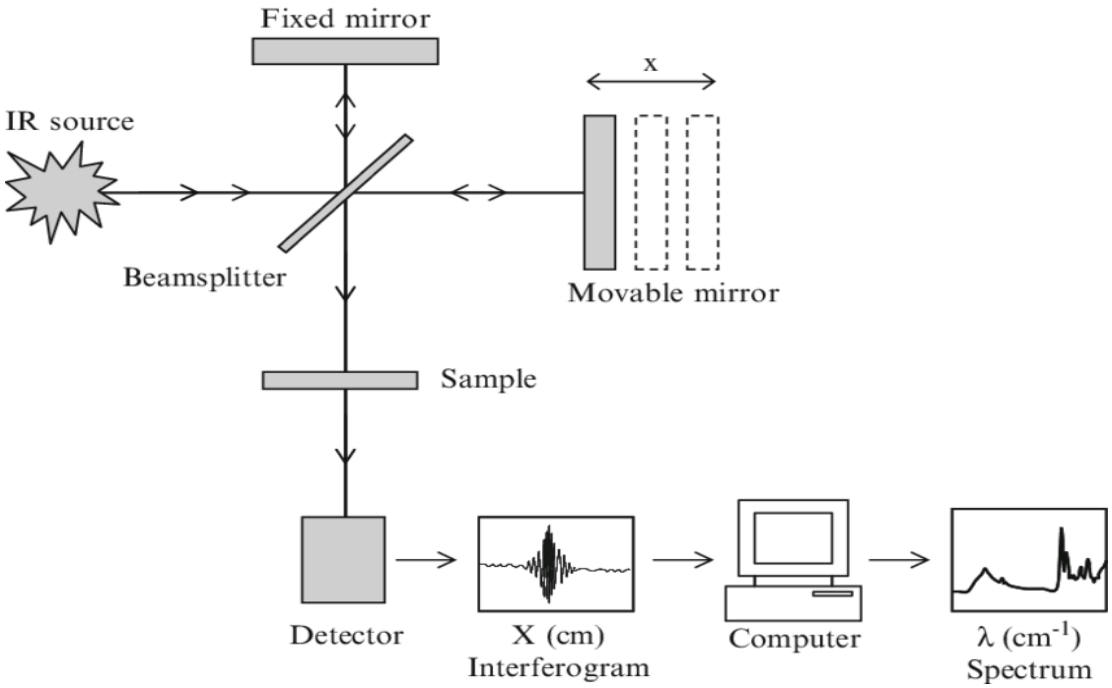


Figure II. 9: The principle scheme of a Fourier transformed spectrometer[13].

II.6.3 Transmission electron microscopy

TEM is a valuable, frequently used, and important technique for the characterization of nanomaterials , used to obtain quantitative measures of particle and/or grain size, size distribution, and morphology . The magnification of TEM is mainly determined by the ratio of the distance between the objective lens and the specimen and the distance between objective lens and its image plane . TEM has two advantages over SEM: it can provide better spatial resolution and the capability for additional analytical measurements . The disadvantages include a required high vacuum, thin sample section , and the vital aspect of TEM is that sample preparation is time consuming. Therefore, sample preparation is extremely important in order to obtain the highest-quality images possible.

The morphology of new synthetic nanoparticles was characterized by transmission electron microscopy (TEM) type JEOL-JEM 2010 operating at 200KV. The measurements of the ZetaSize nano-detector were performed on a Zetasizer Nano-ZS90 instrument.

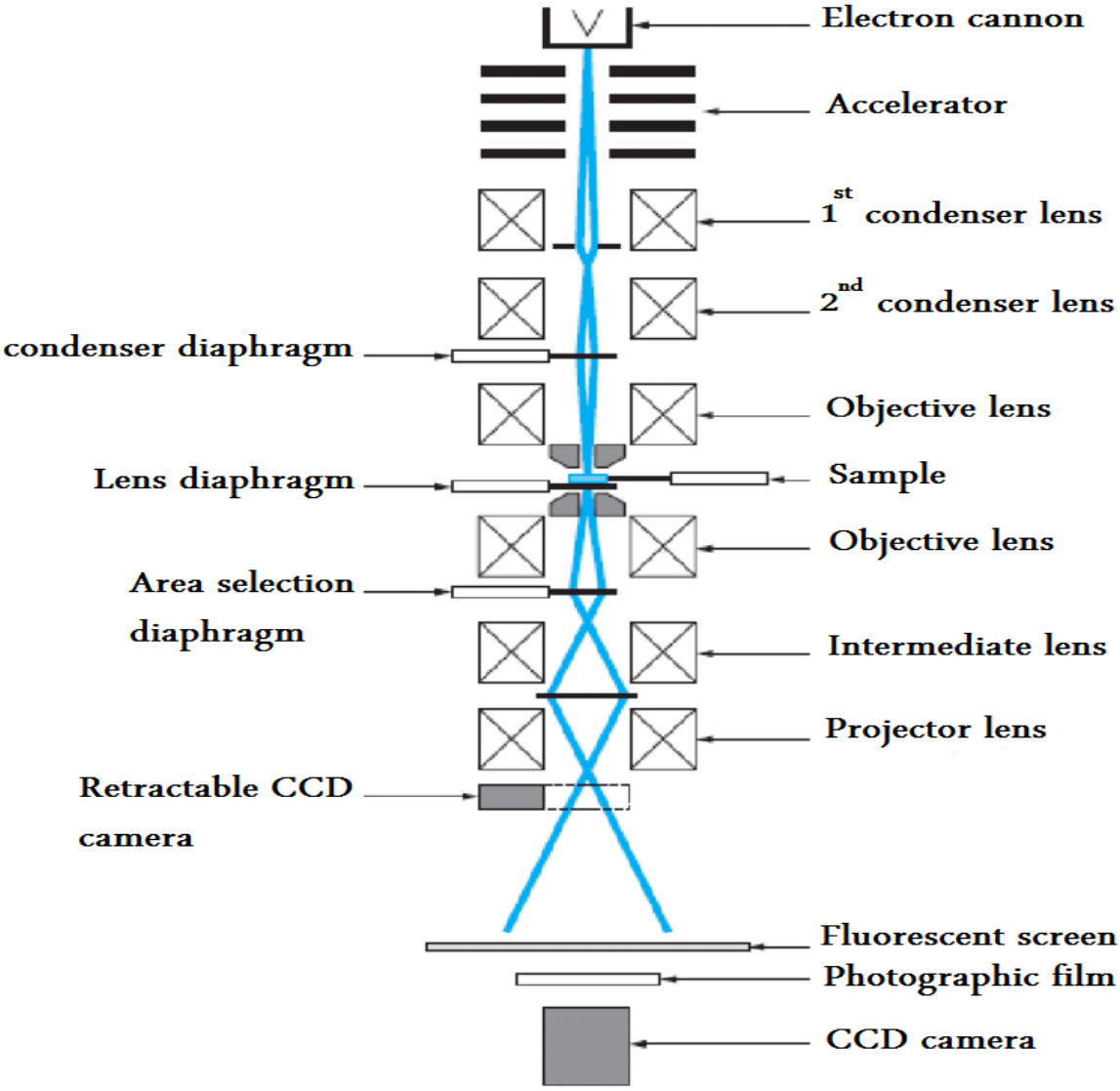


Figure II. 10:Transmission electron microscopy scheme [13].

II.7 Software used :

II.7.1 Origin

Origin is a data analysis and graphics creation software widely used in various fields such as scientific research, industry and education. It provides a comprehensive platform for importing, processing, analyzing and visualizing data, as well as creating high-quality graphics, The software also offers a wide range of graphics options, allowing you to create 2D and 3D graphics with various types of tracks, customizable axes, subtitles, annotations and aesthetic shapes [14].

II.7.2 Chemix

Chemix is an educational app that lets you easily draw lab diagram setups and explain your experiments. It has a large library of highly customisable apparatus and various features to help you draw diagrams with ease [15].

II.7.3 KingDraw

KingDraw App is a free chemical drawing editor that enables users to sketch molecules, reactions, and organic chemistry objects. It supports chemical research, converts chemical structures to IUPAC names, and views 3D models. KingDraw connects Android and iOS devices and PCs, transforming from KingDraw to Office, ChemDraw, and Picture. KingDraw, professional structural formula tool, creates a special work station for chemists[16].

II.8 Conclusion :

Our experience has allowed us to produce silver nanoparticles modified by the amino acid L-Cysteine. We then tested their ability to detect different molecules. The results obtained have been interpreted using characterization techniques (UV-visible and infrared spectroscopy, transmission electron microscopy) which we will interpret in the next chapter.

CHAPTER III

RESULTS AND DISCUSSIONS

III.1 Introduction

In this chapter, we interpret the results obtained before and after the modification of silver nanoparticles using "L-Cys-AgNPs" through several analytical techniques. We will analyze the results from UV-visible spectrum measurements, infrared spectrum, transmission electron microscopy, and colorimetric chiral detection.

III.2. UV-Visible spectrophotometry analysis

The unique attribute of silver nanoparticles is their ability to absorb light within the visible range of the spectrum. This particular absorption band, commonly referred to as the plasmon band, identifies the properties of the nanoparticles. Specifically, the bandwidth and placement provide an overview of the nanoparticles' scattering and size. The absorption band of the silver nanoparticles is detected at approximately 438 nm, while the AgNPs modified by L-cysteine have an absorption band at about 460 nm, which occurs due to the excitation of the plasmon caused by the incident light. The absorption spectra of the two solutions prepared using the procedures described in Chapter II are illustrated in Figure III.1.

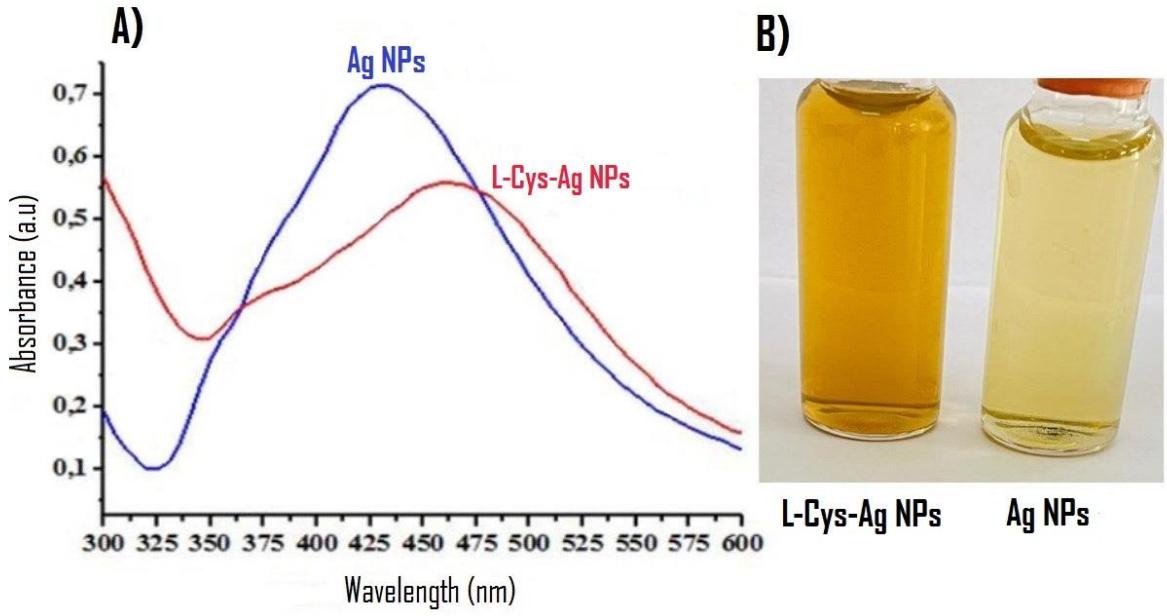


Figure III. 1:(A) UV-Vis spectrum of AgNPs and L-Cys-AgNPs solutions

(B) Photographic images of AgNPs before and after modification by L-Cysteine.

As can be seen from Figure III.2(A), the red spectrum of the L-cysteine modified silver nanoparticle solution shows a maximum absorption band at 460 nm, whereas the UV-visible (blue) spectrum indicates a maximum absorption band at 438 nm for the colloidal silver solution. Photographs of AgNPs, both before and after L-cysteine modification, are shown in Figure III.2(B).

According to the absorption spectrum of silver nanoparticles functionalized with the amino acid, there is a maximum and singular peak due to the resonant excitation of plasmonic oscillations, called surface plasmon resonance. However, compared to the surface plasmon resonance of same-sized AgNPs, the spectrum of the AgNPs modified with L-cysteine shifts by more than 20 nm. This spectral shift is a well-known transition that indicates an interaction between silver nanoparticles and the amino acid L-cysteine. This interaction results in a change in the plasmonic surface of the silver nanoparticles due to an increase in the optical index of the surrounding medium..

III.3 Infrared spectrophotometry analysis

The interaction between silver and the amino acid L-cysteine is verified using infrared spectroscopy. It is important to observe the displacement of the distinct bands assigned to the various functional groups present in the two reagents in order to determine the transparent interaction between the silver nanoparticles and L-cysteine. These functional groups are the sulfhydryl (-SH) and carbonyl groups, which combine to form an amine and a thiol.

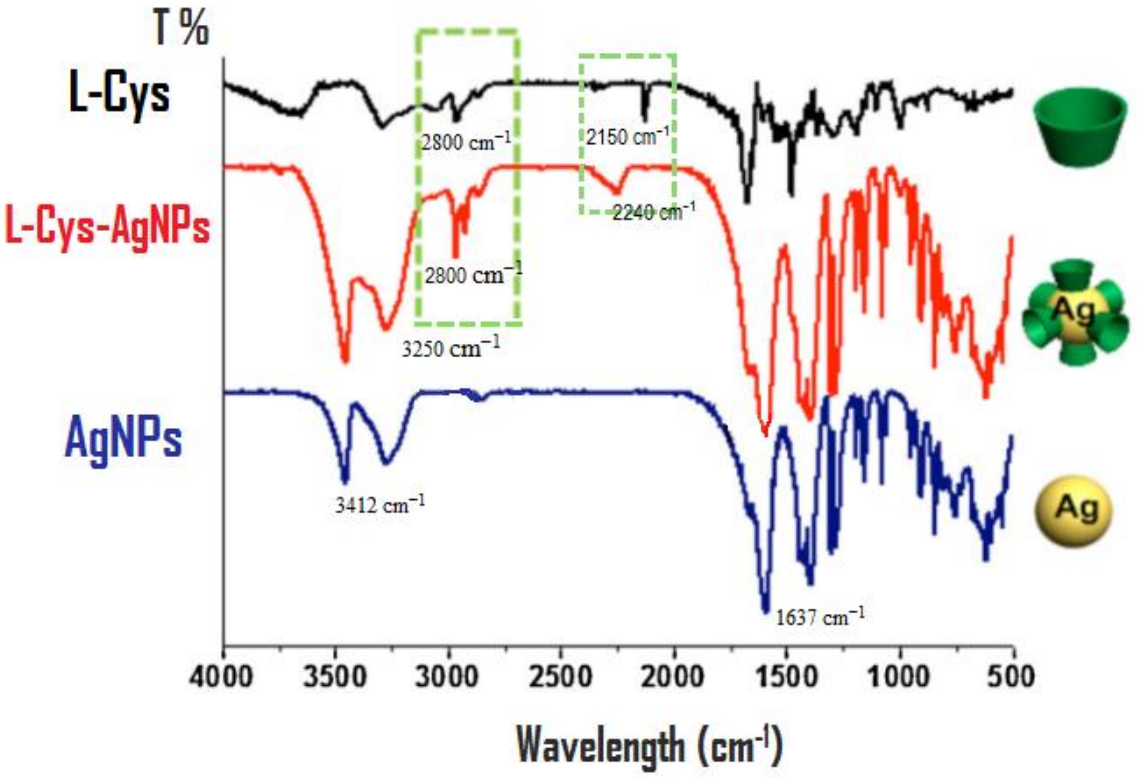


Figure III. 2: Infrared spectra of the compounds L-Cysteine, L-Cys-AgNPs, and AgNPs, respectively.

The typical FT-IR spectra of L-Cysteine-modified AgNPs and AgNPs are shown in the above figure.

In comparison with the IR spectrum of L-Cys and L-Cys -Ag NPs indicates that L-Cys was modified onto the surface of Ag NPs via the interaction of SH and silver. As presented in Fig. III.2, significant features can be seen after Ag NPs coated with L-Cys, the C=CH stretching frequencies from 2150 cm^{-1} exhibited red shift to 2240 cm^{-1} , in the meantime, the absorption peaks are broadened. In addition, a subtle band close to 2800 cm^{-1} is assigned to the group (-SH) of the cysteine molecule .The FT-IR spectra exhibit the stretching modes of O–H (3412 cm^{-1}), C=O (1637 cm^{-1}) and C–O (1070 cm^{-1}) bonds indicating the successful modification of L-Cys.

The final result indicates that the plasmonic surface modification of silver nanoparticles is successful and that there is an interaction between the amino acid L-cysteine and the nanoparticles.

III.4 The size of the nanoparticles L-Cys-AgNPs

The use of transmission electron microscopy (TEM) is essential for the study of materials at the nanoscale. As shown in Figure III.3, AgNPs modified by L-cysteine were evenly distributed throughout the solution. This observation confirms that the amino acid L-cysteine was successfully implanted on the surface of the AgNPs, resulting in an exceptional degree of monodispersity and uniformity. The size of the modified silver nanoparticles was around 9 nm.

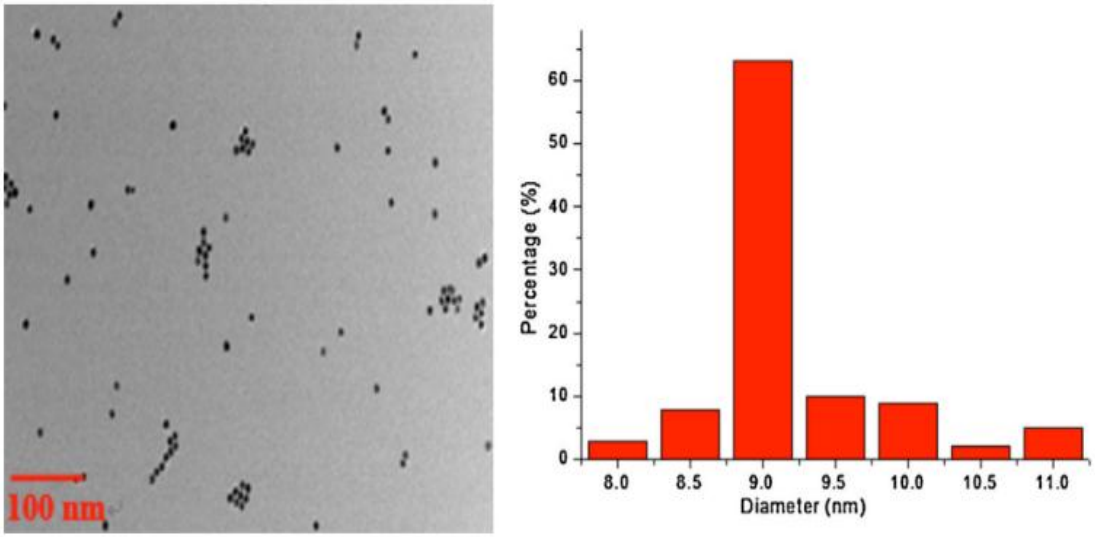


Figure III. 3: TEM of L-Cys-AgNPs and size distribution of L-Cys-AgNPs,the bars 100 nm.

III.5 Schematic mechanism of silver nanoparticles functionalized with L-Cysteine

Research has demonstrated that both ionic silver and silver nanoparticles (AgNPs) exhibit strong attraction towards both inorganic and organic solvent-saturated compounds. It has been observed that AgNPs interact with weak amino acids, such as cysteine, through the thiol group present in the molecule. Moreover, several studies have indicated that proteins can also bind to AgNPs via their thiol groups. Hydrogen bonding facilitates the attachment of the carboxylic acid group to the nanoparticle, acting as a bridging unit. This design is believed to enhance the stability of the complex.

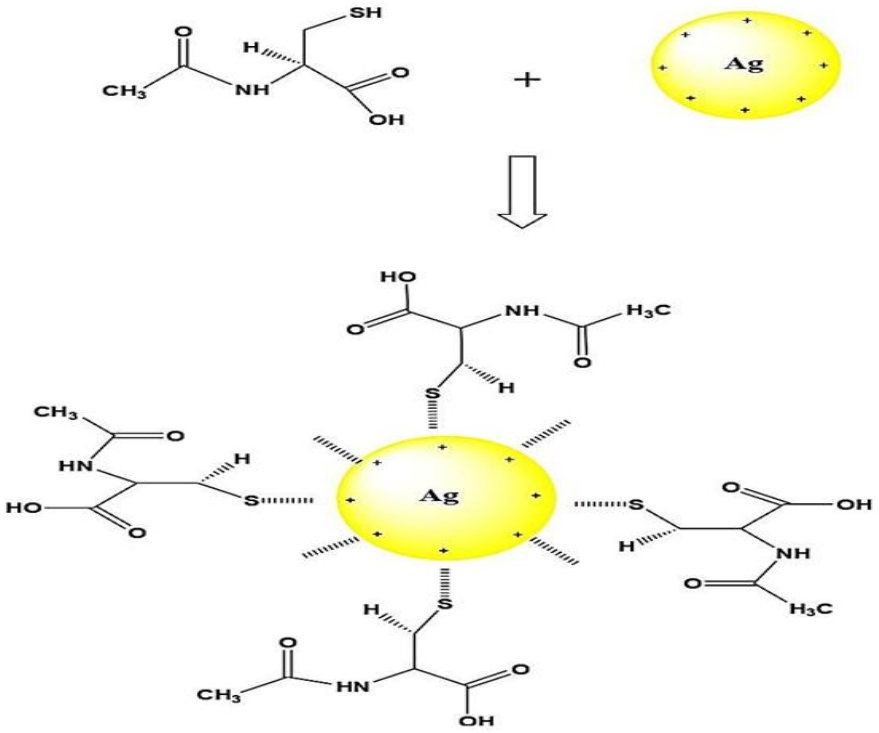


Figure III. 4:The possible interaction mode between L-Cysteine and AgNPs.

III.6. Stability of AgNPs functionalized with L-Cysteine

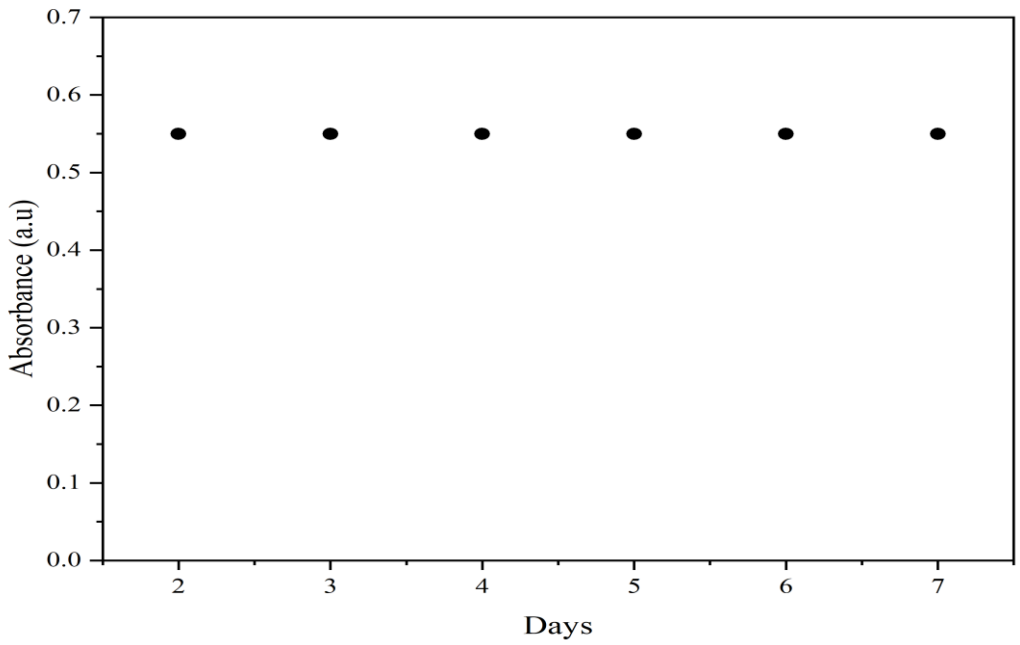


Figure III. 5:Stability of L-Cys-AgNPs. The UV-Vis absorption spectrum of L-Cys-AgNPs recorded at different times.

Figure III.5 shows the stability of L-Cys-AgNPs over time. The UV-Vis absorption spectroscopy of silver nanoparticles modified by L-cysteine on their surface, obtained at various time points, is displayed in the figure. During the first seven days, there was no discernible change in the shape, position, or symmetry of the UV-Vis absorption peak for L-Cys-AgNPs. Furthermore, no noticeable color change occurred throughout this period. The study's results indicate that L-Cys-AgNPs, without additional protection, exhibit reasonable stability at room temperature

Figure III.6 illustrates how L-Cys-AgNPs sensors respond to pH shown in figure below .The procedure involved calibrating the acidic medium (a solution of silver nanoparticles surface-modified with L-Cysteine) by adding an HCl solution, and the basic medium (a solution of NaOH). Absorption measurements were then taken from these solutions. The HCl and NaOH solutions used had a concentration of 10^{-3}M.

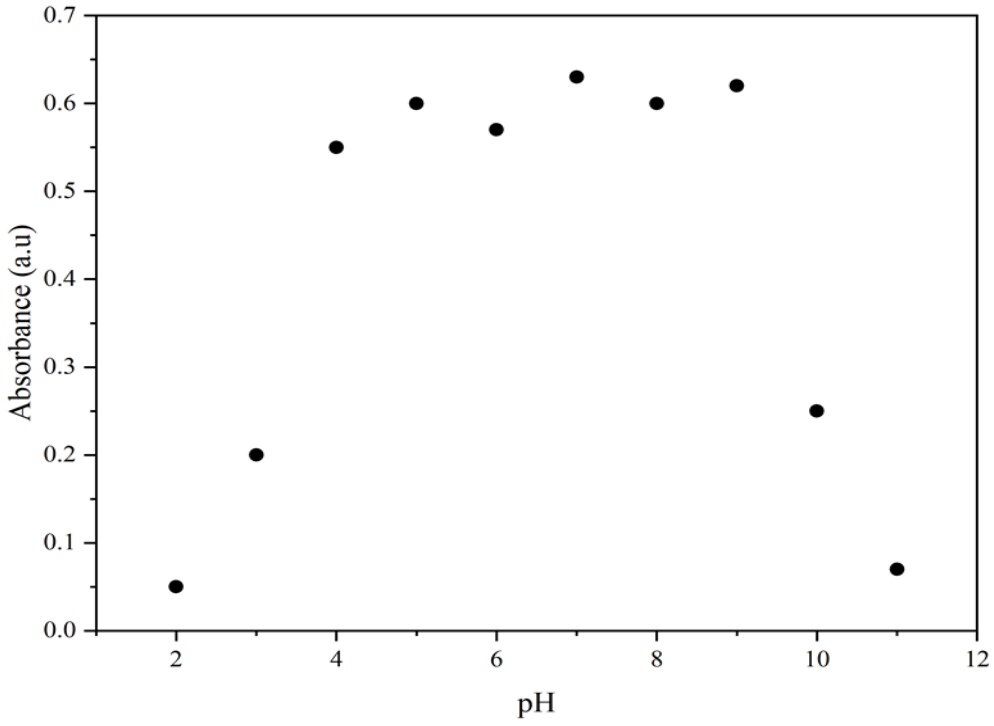


Figure III. 6: The effect of pH on the UV-Vis absorption spectrum of L-Cys-AgNPs

According to the graph, we can observe that the freshly synthesized L-Cys-AgNPs are quite stable within the pH range of 4.0 to 9.0. This stability can be attributed to the presence of sodium citrate in the silver nanoparticles, which acts as both a chemical reducing agent and a stabilizer. The results indicate that L-Cys-AgNPs exhibit high stability under neutral conditions.

III.7. Colorimetric detection

III.7.1. The UV-visible spectrum for colorimetric detection

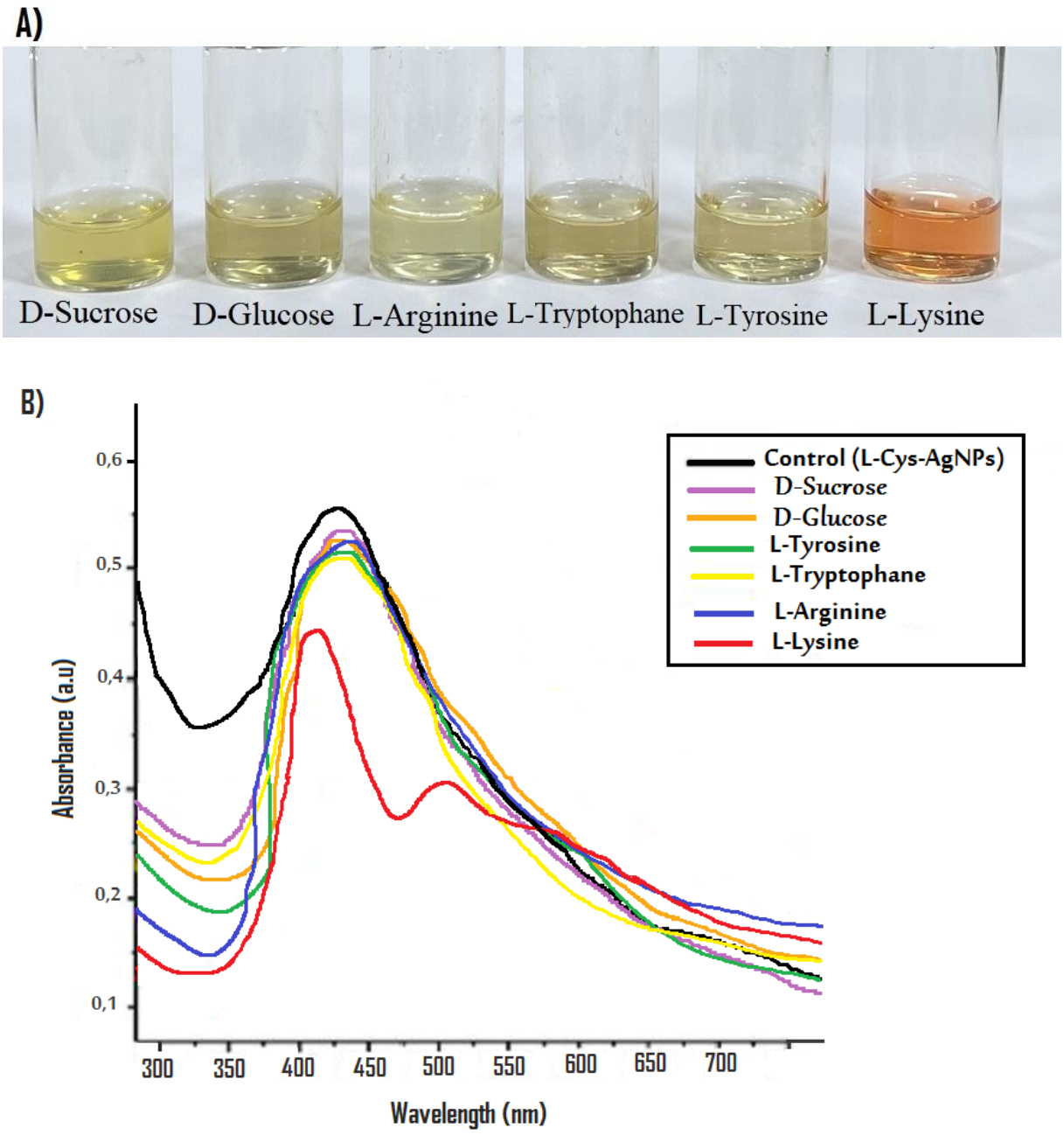


Figure III. 7:(A) photographic images and (B) UV-Vis spectrum of the L-Cys-AgNPs solution in the presence of 10 mM of the different prepared solutions respectively.

In Figure III.7(A), a color change is observed specifically in the vial containing L-Lysine. Additionally, there is a noticeable variation in the spectrum of L-Lysine compared to the control solution (L-Cys-AgNPs). In contrast, Figure III.7(B) shows that the spectrum of the other sample remains almost stable without any change

Figure III.7 (A) shows a sharp color change from yellow to brick red after only 5 minutes in the presence of the L-Lysine chiral amino acid solution, which corresponds to a dramatic decrease in absorption intensity and wavelength shift from 440 to 500 nm.

However, the other solutions showed no noticeable effect on color and UV-Vis absorption, demonstrating that L-Cys-AgNPs selectively respond to L-Lysine. This response can be attributed to the aggregation induced by L-Lysine. The mechanism of this phenomenon is illustrated in Figure III.8

III.7.2. Schematic mechanism of colorimetric detection of L-Lysine

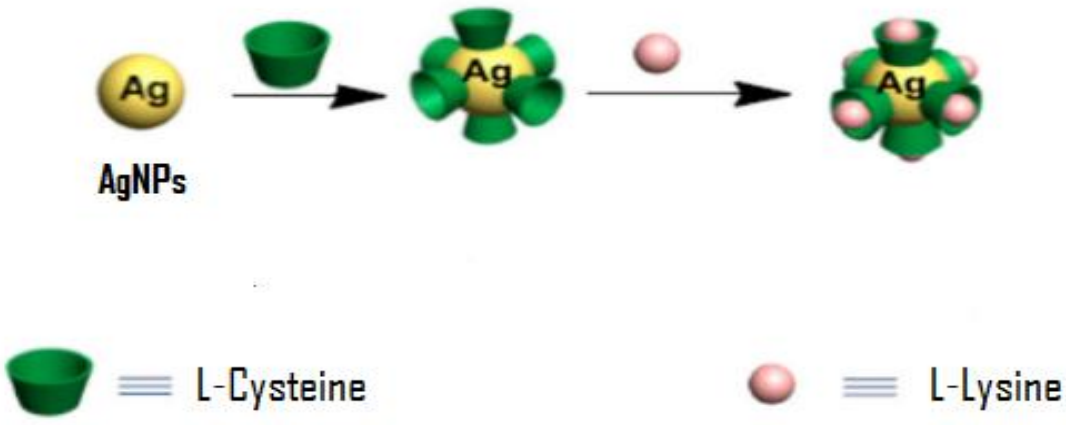


Figure III. 8: Schematic representation of Ag NPs coated with L-Cysteine for detecting L-lysine.

L-Cystine-modified silver nanoparticles (L-Cys-AgNPs) can capture and cluster L-Lysine molecules, demonstrating high sensitivity to the presence of L-Lysine. This results in increased particle size and heavy aggregation, proving effective and sensitive selectivity for L-Lysine detection.

III.7.3. TEM images of L-Cys-AgNPs colloidal solutions before and after L-Lysine detection

To justify the color change and UV-Vis absorption, we analyzed TEM images before and after the addition of L-Lysine. The illustration below depicts the structure of silver nanoparticles modified by L-cysteine before and after testing for L-lysine detection using transmission electron microscopy (TEM).

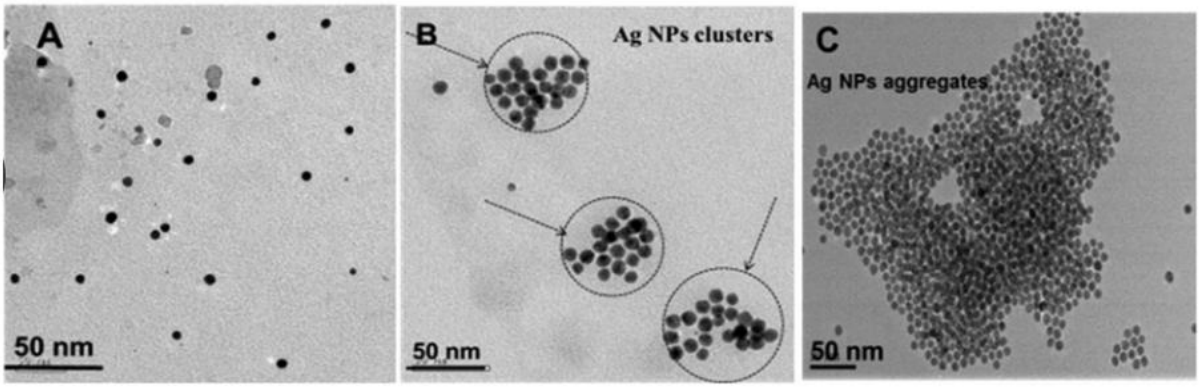


Figure III. 9:TEM images of L-Cys-AgNPs solutions before and after L-Lysine detection with 50 nm scale bars.

In the water solution depicted in Figure III.9: (A) Ag NPs decorated with L-Cys are highly dispersed and uniform. (B) Upon addition of L-Lysine, the nanoparticles self-assemble into clusters, indicating successful attachment of L-Lysine onto L-Cys. (C) The Ag NP clusters appear much larger with broader size aggregates; scale bars in both images are 50 nm.

The nanoparticles were reduced to form silver nanoparticle clusters. These results further confirm that the formation of silver nanoparticle clusters is due to the host-guest inclusion complexation between L-Cys and L-Lysine motifs.

To explore the color change and differences in UV-vis absorption, we observed TEM images of L-Cys-Ag NPs before and after adding L-Lysine. The TEM image (Fig. III.9 C) shows that after the addition of L-Lysine, the Ag NP clusters were much larger with broader size aggregates. This aggregation resulted in a yellow-to-red color change, reflecting the L-Lysine-induced aggregation of functional Ag NPs. The proposed mechanism of this phenomenon is illustrated in Scheme III.8.

III.7.4. Sensitivity and detection limit of L-Lysine

A simple method was used to determine the sensitivity of direct colorimetric visualization of L-lysine, and the results are presented in Figure III.10. The lowest concentration of aqueous lysine solution that could be identified by color change was determined using 1.5 ml of L-Cys-AgNPs. L-lysine solutions at various concentrations (from 10^{-2} M to 10^{-7} M) were prepared, and 0.5 ml of each solution was added to different vials. The solutions were analyzed using UV-Vis spectroscopy after five minutes of incubation

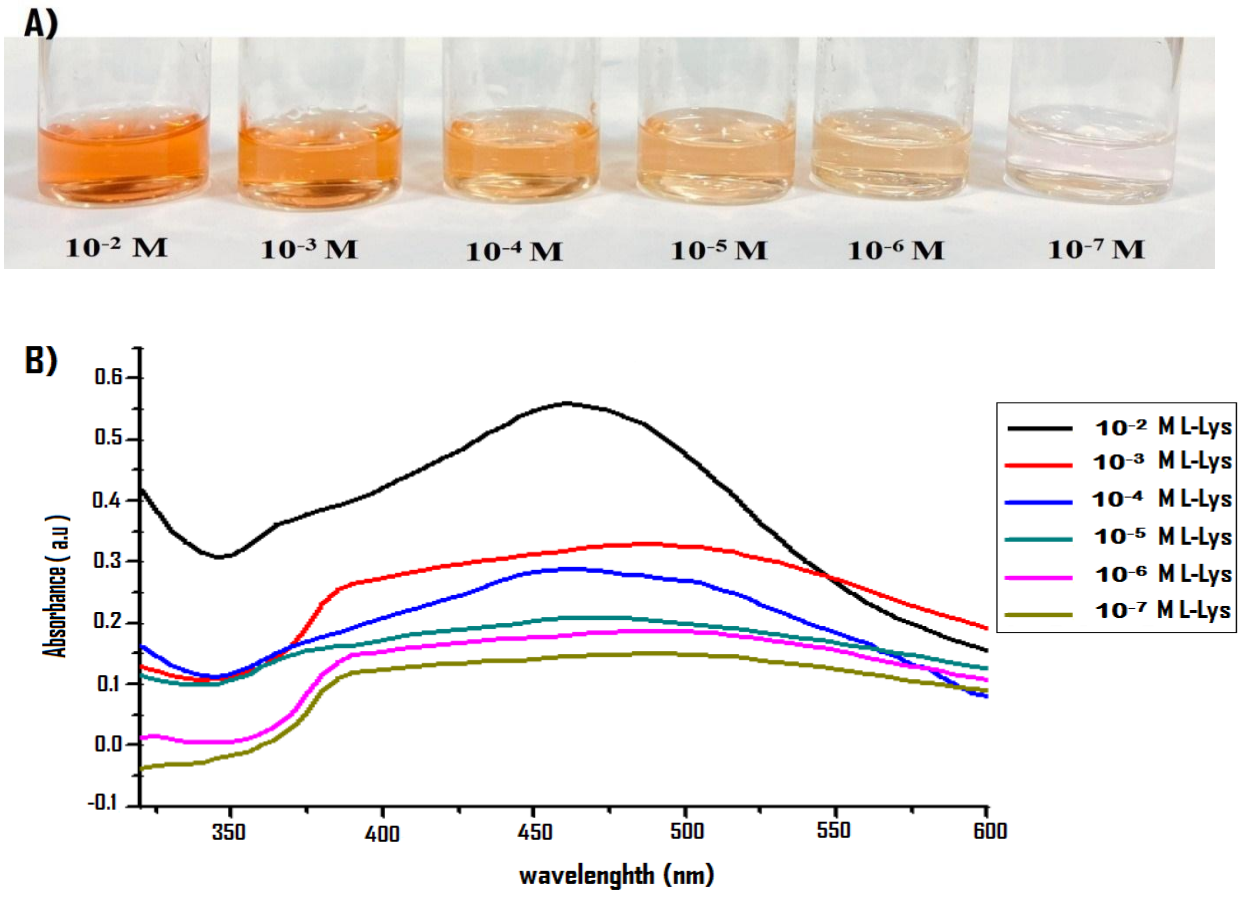


Figure III. 10:(A) The change in color of the L-Cys-AgNPs solution following the addition of various L-Lysine concentrations (10^{-2} M to 10^{-7} M) and (B) The UV spectra of each of the L-Lysin concentrations (10^{-2} M to 10^{-7} M).

When L-Lysine is added to the colloidal solution of silver nanoparticles synthesized by L-Cysteine at room temperature, a noticeable change in color occurs, as depicted in Figure III.10 (A). Specifically, as the L-Lysine concentration decreases from 10^{-2} M to 10^{-7} M, the color of the L-Lys-L-Cys-AgNPs solution changes. Figure III.10 (B) displays the UV-Vis spectrum of different concentrations of L-Lysine (ranging from 10^{-2} M to 10^{-7} M) in the colloidal solution of silver nanoparticles synthesized by L-Cysteine at room temperature.

The addition of lysine results in an increase in the size of the silver clusters, leading to large aggregates and a change in UV-Vis absorption, which also explains the observed color changes. Upon examining the spectrum, a broad band is observed around 470 nm. This band shows decreased intensity compared to the isolated colloids in solution, as demonstrated by the spectrum of the L-Cys-AgNPs solution. Furthermore, it is observed that the curve stabilizes when the concentration of L-lysine exceeds 10^{-7} M. Based on these findings, it can be concluded that the limit of L-lysine detection by the colorimetric method is 10^{-7} M.

III.8. Nanosensors based on L-Cysteine-AgNPs for the selective detection of L/D Lysine

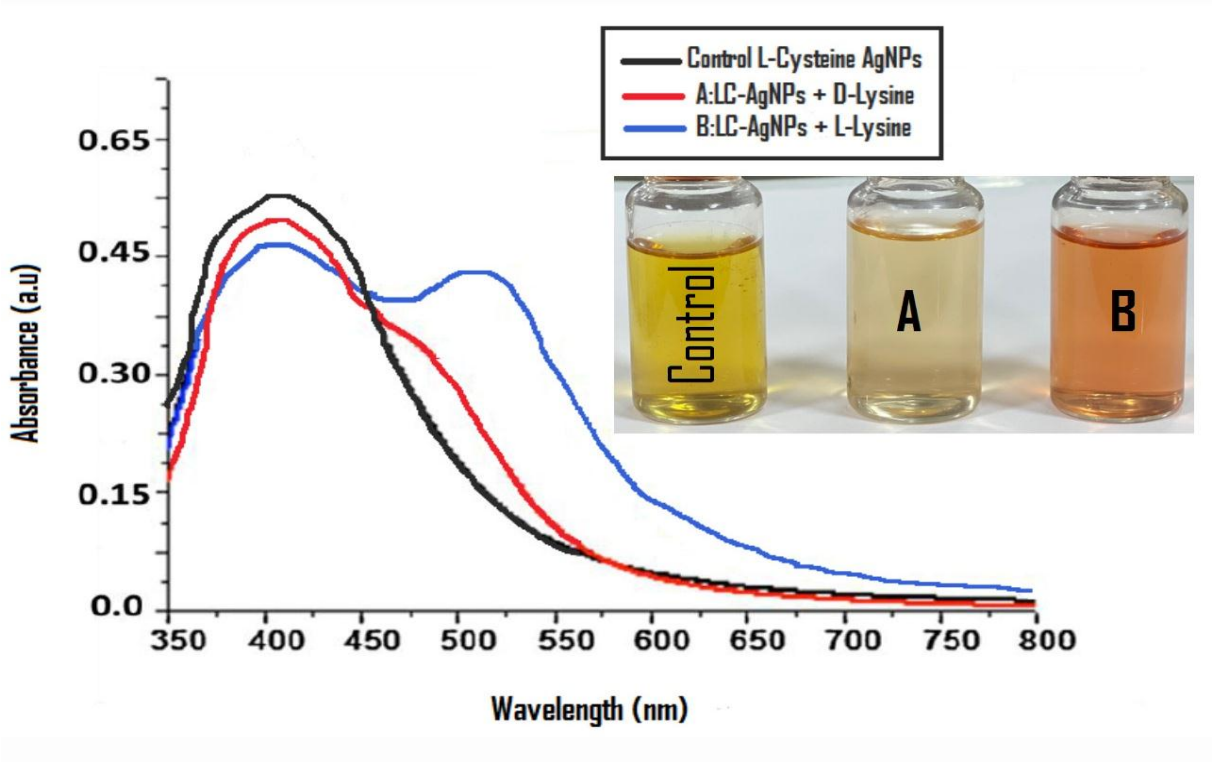


Figure III.11: The UV-Visible spectra of the L-Cysteine-AgNPs system change when the amino acid L/D Lysine is added, respectively.

To investigate the molecular recognition capability of L-Cysteine-AgNPs, several amino acids at the same concentration (Figure III.11) were individually added to the L-Cysteine-AgNPs solution. Among all the tested amino acid derivatives, only D/L Lysine caused a slight color change and altered the UV-vis absorption spectra of L-Cysteine-AgNPs. This suggests that the system can preliminarily differentiate between the chiral derivatives of lysine. As depicted in the figure, the color of the L-Cysteine-AgNPs system changes slightly upon the addition of D-Lysine, while the addition of L-Lysine results in a new UV-vis absorption peak at 525 nm, indicating a strong interaction between L-Cysteine-AgNPs and L-Lysine

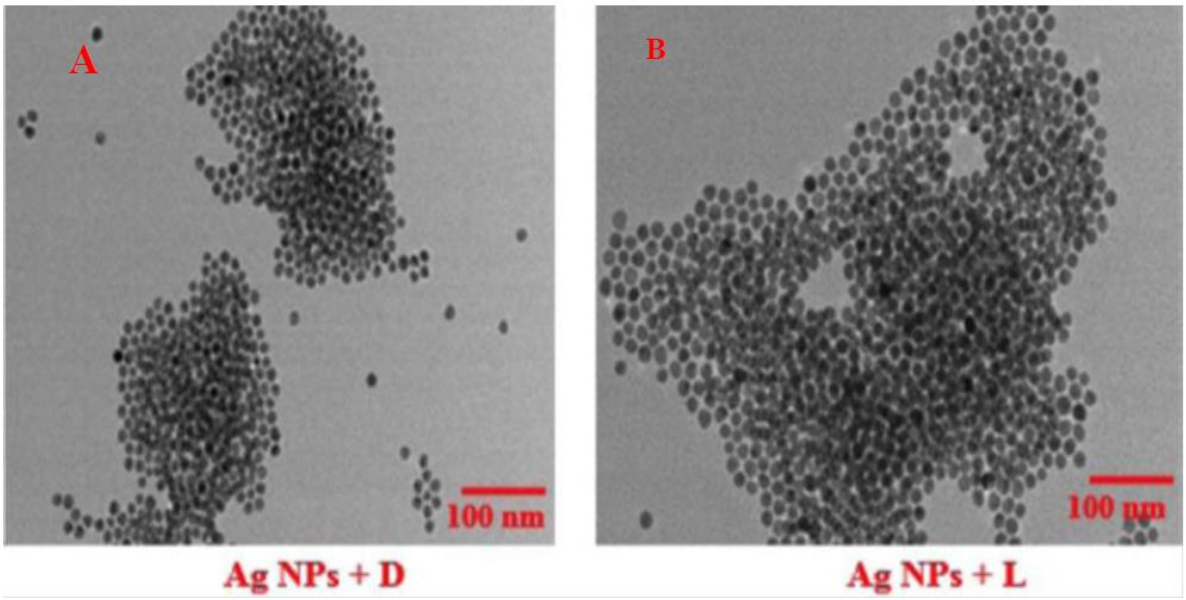


Figure III.12: TEM images of L-Cysteine-AgNPs with the addition of D-Lysine (A), and L-Cysteine-AgNPs with the addition of L-Lysine (B).

Moreover, the L-Cysteine-AgNPs sensors were also characterized by transmission electron microscopy (TEM), as shown in Figure III.12, which is considered an effective method to enhance selective sensitivity. For this system, as presented in Figure III.12, the Ag NPs modified by L-Cysteine were found to be highly sensitive to the presence of L-Lysine. From Fig. B, when L-Lysine was added to the silver nanoparticles modified by L-Cysteine, the size of the resulting particles was relatively larger due to aggregation compared with Fig. A.

Conclusion

Silver nanoparticles exhibit strong visible absorptions, as determined by UV-Vis characterization to acquire their optical absorption spectrum. The modified colloids showed the highest absorption bands at 438 nm and 460 nm, respectively.

AgNPs modified by L-cysteine were found to be remarkably stable for up to 7 days, with an extraordinary stability range between 0.4 and 0.9. Transmission electron microscopy (TEM) revealed that these modified nanoparticles possess a unique shape conducive to the selective detection of L-Lysine.

Using optical colorimetric detection, AgNPs modified with L-Cysteine were synthesized. The UV-Vis absorption spectrum underwent noticeable alterations, resulting in a color change of the solution from yellow to red due to the aggregation of AgNPs induced by L-Lysine.

GENERAL CONCLUSION

General conclusion

Our primary objective is to master the synthesis of stable silver nanoparticles (AgNPs) coated with the amino acid L-cysteine (L-Cys) on their surface, aiming to achieve silver nanoparticles functionalized with L-Cys. These nanoparticles are capable of colorimetrically recognizing and enriching L-lysine (L-Lys). We chose the chemical reduction method due to its adaptability to our study needs. Nanoparticles modified with L-Cys have been used as highly selective surfactants in optical sensors for detecting L-lysine. Silver nanoparticles, owing to their unique properties in terms of size, shape, and surface characteristics, are crucial for understanding their synthesis, controlling their fabrication, and exploring their potential applications in fields such as biology, electronics, and electro-optics.

Silver nanoparticles possess unique properties in terms of size, shape, and surface characteristics, which are essential for understanding their synthesis and controlling their applications in fields such as biology, electronics, and electro-optics. Optimizing synthesis conditions, including reagent concentrations and temperatures, is critical for obtaining uniform and stable silver nanoparticles.

The synthesis involved using silver nitrate as a precursor and sodium citrate as a reducing or stabilizing agent. The formation of nanoparticles of different sizes was evidenced by variations in the color of the solutions. Synthetic silver nanoparticles were characterized using infrared (IR) and UV-visible (UV-Vis) spectroscopy, as well as transmission electron microscopy (TEM). UV-Vis spectroscopy revealed the absorption spectra of silver colloids in aqueous media, indicating plasmonic resonance. Infrared spectroscopy confirmed the interaction between silver and L-cysteine, with a weak band attributed to the (-SH) group of L-cysteine suggesting a strong covalent bond (Ag-S), affirming the successful modification of silver with L-cysteine. TEM provided morphology details of silver nanoparticles modified by L-cysteine, showcasing their agglomeration potential for selective detection of L-lysine in aqueous solutions with high specificity.

In conclusion, we synthesized chiral Ag nanoparticles coated with L-cysteine for enantio-selective recognition and enrichment of L-lysine through nanoparticle aggregation. This method proves cost-effective and less time-consuming compared to previous procedures. UV-Vis and infrared spectroscopy detected L-Lys accompanied by aggregated nanoparticles

in solution, suggesting potential applications in high-performance enantiomer separation of amino acids.

Future directions in silver nanoparticle synthesis involve integrating smart technologies and tailoring nanoparticles for specific applications such as targeted drug delivery and advanced detection devices. Continued research is essential to fully explore the potential of these nanoparticles and develop novel applications.

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عنوان المذكرة : **الكشف اللوني الميراثي للأحماض الأمينية باستخدام الجسيمات النانوية الفضية المطلية بالإيل-سيستين.**

اللقب: بن نعيجة **الإسم**: إبتسام **المؤطر:** بوصوار إيمان

معاش فاطنة

ملخص :

تُعرَّف الجسيمات النانوية عمومًا على أنها كيانات تقيس من 1 إلى 50 نانومتر. تُظهر جسيمات الفضة على مقياس النانو خصائص فريدة تجعلها مفيدة وتلعب دورًا أساسيًا في جميع المجالات العلمية والتكنولوجية مثل علم الأحياء والطب والإلكترونيات الضوئية. في هذه الدراسة، قمنا بإنشاء مستشعر لوني بسيط وحساس للكشف عن الإيل-لايسين. تم تزيين الجسيمات النانوية الفضية بحمض أميني الإيل-سيستين. أدى إضافة الإيل-لايسين إلى تكوين تجمعات كبيرة من الجسيمات النانوية الفضية، مما تسبب في تغييرات في اللون وامتصاص الأشعة فوق البنفسجية-المرئية. تُظهر هذه المجسّة حساسية وانتقائية متزايدة مقارنةً بالطرق البصرية الأخرى المذكورة.

الكلمات المفتاحية: الجسيمات النانوية ، المقياس النانوي، الجسيمات النانوية الفضية، الإيل-ليسين ، الإيل-سيستين ، الحساسية، الانتقائية.

Thesis title : Chiral colorimetric detection of amino acids based on L-Cysteine capped silver nanoparticles.

Name : BENAIDJA MAACHE **First name : Ibtissam Fatna** **Directed by : BOUSSOUAR Imene**

Abstract :

Nanoparticles are generally defined as entities measuring from 1 to 50 nm. Silver particles at the nanoscale exhibit unique properties that make them useful and play an essential role in all scientific and technological fields such as biology, medicine, and optoelectronics. In this study, we created a simple and sensitive colorimetric sensor to detect L-lysine. Silver nanoparticles were decorated with the amino acid L-cysteine. The addition of L-lysine led to the formation of large aggregates of silver nanoparticles, causing changes in color and UV-Visible absorption. This probe demonstrates increased sensitivity and selectivity compared to other optical methods mentioned.

Key words: Nanoparticles, silver nanoparticles , nanoscale, L-Lysine, L-Cysteine , sensitivity ,selectivity.

Titre du mémoire : Détection colorimétrique chirale d'acides aminés à base de nanoparticules d'argent enrobées de L-cystéine.

Nom: BENAIDJA MAACHE **Prénom: Ibtissam Fatna** **Encadreur: BOUSSOUAR Imene**

Résumé :

Les nanoparticules, généralement définies comme des entités mesurant de 1 à 50 nm. Les particules d'argent et l'échelle nanométrique présentent des propriétés uniques qui les rendent utiles et jouent un rôle essentiel dans tous les domaines scientifiques et technologiques tels que la biologie, la médecine, optoélectronique. Au cours de cette étude, nous avons créé un capteur colorimétrique simple et sensible pour détecter la L-Lysine. On avait orné les nanoparticules d'argent de l'acide aminé L-Cystéine. . L'ajout de L-Lysine a entraîné la formation de larges agrégats de nanoparticules d'argent, provoquant ainsi des changements de couleur et d'absorption UV-Visible. Cette sonde présente une sensibilité et une sélectivité accrues par rapport aux autres méthodes optiques mentionnées.

Mots clés : nanoparticules, nanoparticules d'argent, l'échelle nanométrique , L-Lysine, L-Cystéine, sensibilité , sélectivité.