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Nanoparticles for Drug Delivery: Recent Advances

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Abstract. *Silver nanoparticles (AgNPs) have demonstrated numerous physicochemical, biological, and functional properties suitable for biomedical applications, including anticancer and drug carrier properties. In the present study, the antibiotic, Levofloxacin (Levo), was loaded onto AgNPs, which were synthesized via the chemical reduction method, and evaluated as antioxidants and anticancer agents. The characterized XRD and FE-SEM analyses were 10-40 nm. The antioxidant potentials of AgNPs and their conjugates, tested via their 1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging ability Overall, according to the results obtained in the present study, the new nanocomposite, AgNPs-Levo, showed the highest antioxidant activity and anti-cancer activity. The preparation has high clinical potential for prospective use as an Anticancer agent.*

CHAPTER ONE

INTRODUCTION

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INTRODUCTION

1. Nanoparticles:

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix.

Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly (ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes (Langer R et al., 2000; Bhadra D et al., 2002; Kommareddy S et al., 2005; Lee M et al., 2005).

The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, targeting to site of action and reduction toxicity or side effects, their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties (Vila A et al., 2002; Mu L et al., 2003).

The advantages of using nanoparticles as a drug delivery system include the following:

1. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
2. They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.
3. Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.
4. Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
5. The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.

In spite of these advantages, nanoparticles do have limitations. For example, their small size and large surface area can lead to particle- particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms. In addition, small particles size and large surface area readily result in limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available. The present review details the latest development of nanoparticulate drug delivery systems, surface modification issues, drug loading strategies, release control and potential applications of nanoparticles.

2. Synthesis of nanoparticles:

Nanoparticles can be synthesized chemically or biologically. Many adverse effects have been associated with chemical synthesis methods due to the presence of some toxic chemical absorbed on the surface. Eco friendly alternatives to Chemical and physical methods are biological ways of nanoparticles synthesis using microorganisms (Klaus T et al., 1999; Konishi Y et al., 2007), enzymes (Willner I et al., 2006), fungus (Vigneshwaran N et al., 2007), and plants or plant extracts (Shankar S.S et al., 2004; Ahmad N et al., 2011). The development of these eco-friendly methods for the synthesis of nanoparticles is evolving into an important branch of nanotechnology especially silver nanoparticles, which have many applications (Armendariz V et al., 2002; Kim B.Y et al., 2010; Kyriacou S.V et al., 2004). The nanoparticles are synthesized by various methods that are categorized

into top-down or bottom-up method. A simplified representation of the process is presented in figure (1-1).

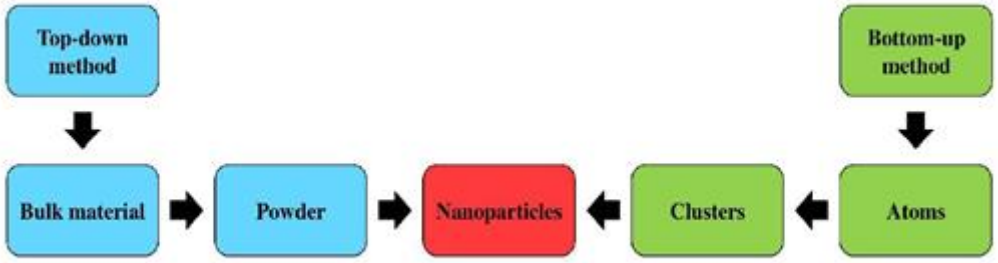

Figure (1-1): Synthesis process

1.2.1 Top-down:

Top-down approach involves the breaking down of the bulk material into nanosized structures or particles. Top-down synthesis techniques are extension of those that have been used for producing micron sized particles. Top-down approaches are inherently simpler and depend either on removal or division of bulk material or on miniaturization of bulk fabrication processes to produce the desired structure with appropriate properties. The biggest problem with the top-down approach is the imperfection of surface structure. For example, nanowires made by lithography are not smooth and may contain a lot of impurities and structural defects on its surface. Examples of such techniques are high-energy wet ball milling, electron beam lithography, atomic force manipulation, gas-phase condensation, aerosol spray, etc.

Mechanicanosynthetic Methods, Mechanical methods offer the least expensive ways to produce nanomaterials in bulk. Ball milling is perhaps the simplest of them all. Ball milling produces nanomaterials by mechanical attrition in which kinetic energy from a grinding medium is transferred to a material undergoing reduction. Compaction and consolidation are an industrial scale process wherein nanomaterials are "put back together" to form materials with enhanced properties. Metallic alloys can be made this way. Many top-down mechanical methods are utilized by industry.

Thermal methods form a nebulous category and we try and focus on those that provide heat to a fabrication process. Of these, electrospinning is a means to form nanothread materials. High energy methods are those that require an excessive input of energy– whether in the form of heat, electricity or solar energy. Arc discharge was the first controlled means of making carbon nanotubes. Laser ablation and solar flux also work well. The problem is control of quality and potential upscale. We include plasma methods in this category. Plasmas are created in high-energy situations (high potential bias, etc.). The problem with this and other high-energy methods is upscale potential– with the possible exception of solar flux methods as sunlight is easily available. Top-down chemical fabrication methods are always easy to upscale and many, such as anodizing, are widespread industrial processes. Lithographic methods, as we all know quite well, although energy intensive and requiring expensive equipment and facilities, are top-down methods capable of producing for the most part micron-sized features. Lithography is the means of making printed circuits and computer boards for several decades now. The push to miniaturize in the future is a costly venture as more powerful sources (high energy electron beams and shorter wavelength sources), support equipment and facilities are required. Nanoimprint lithography (NIL) is lithography but not according to typical standards. It is more like template synthesis. A template material is made first and then stamped into a soft polymeric material to form a pattern. The template stamp is formed by top-down method as is the stamped material. Nanosphere lithography utilizes latex spheres that form a templated matric. So, we can call these techniques template process as well.

1.2.2 Bottom-up:

Bottom-up technique is an approach to synthesize structures or nanomaterials made atom-by-atom or molecule-by-molecule. The atoms/molecules are combined by means of covalent or non-covalent bonds. This technique is widely used in nature to form functional nanomaterials and can be

manipulated easily by utilizing the chemistry of materials. For example, Chemical Vapor Deposition (CVD) is a bottom-up technique in which two (or more) molecules react in the vapor phase inside a CVD reactor leading to formation of nanomaterial over a substrate. The synthesis or formation of the desired material can be preferred by manipulating the pressure and temperature conditions. More examples of the bottom-up techniques are self-assembly (micelles), molecular beam epitaxy (MBE), etc.

Bottom-up methods start with atoms or molecules to form nanomaterials. Chemical vapor deposition is a gas-phase process by which reactive constituents react over a catalyst or pre-templated surface to form nanostructure materials. The economical synthesis of carbon nanotubes is by CVD. Precursors in the form of methane or acetylene or other carbon source gases are passed over Co, Fe or Ni catalyst. Once decomposed into carbon, nanotubes are formed by the catalyst particle. Atomic layer deposition is an industrial process that is capable of coating any material, regardless of size, with a monolayer or more of a thin film. Molecular beam epitaxy and MOCVD are other industrialized processes that are considered to be bottom-up.

Liquid phase methods are also numerous. It is within the liquid phase that all of self-assembly and synthesis occurs. Liquid phase methods are upscalable and low cost.Electrodeposition and electroless deposition are very simple ways to make nanomaterials (dots, clusters, colloids, rods, wires, thin films).

Anodizing aluminium to make a porous oxide structure is a simple way to make nanomaterials. The porous structure is a nanomaterial as well as any material synthesized within. Porous membranes are in many ways the ultimate template. A new generation of nano bottom-up methods have made the scene. Many of the new methods are both inexpensive and offer high throughput. Disadvantages include establishment of long-range order. The new methods include nanolithography (dip pen method) and nanosphere lithography (H. Cheng et al., 1995; S. Ge, X. Shi et al., 2009).

1.3 Synthesis of silver NPs:

Ag NPs have been synthesized using various methods, which can be classified as gas or aerosol, solid, and liquid-phase routes. Both chemical and physical synthesis methods for Ag NPs are well known (De Matteis, V et al., 2018). In recent years, green synthetic pathways have also been proposed (Vijayaraghavan, K et al., 2010). These green processes reduce the generation of harmful byproducts that damage the environment. They also allow for an efficient resource-saving synthesis.

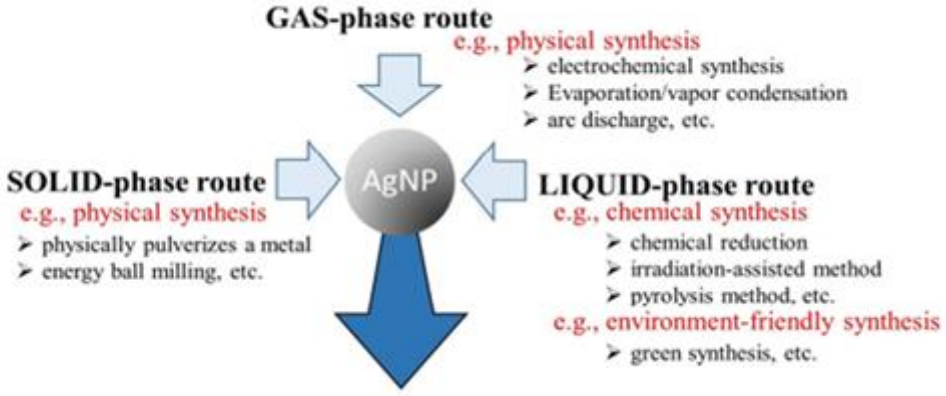

Figure (1-2): Synthesis of AgNPs

The green synthesis method based on green chemistry programs is known as the representative environment-friendly synthesis method. To avoid the use or discharge of hazardous chemical substances to a considerable extent during the synthesis of chemical compounds, green chemistry programs were proposed by the Environmental Protection Agency of the United States (EPA) in 1990. Then, in 1998, Anastas and Warner published the “Twelve Principles of Green Chemistry”, summarizing the concept of green chemistry (Anastas, P.T et al., 1998). Since then, policies concerning the handling of environment-friendly chemical substances have been announced

worldwide. Moreover, green sustainable chemistry advocates for resource savings by recycling, which is not necessarily covered by green chemistry. This consideration has also become widespread in the materials science field, and reports on the green synthesis of Ag NPs have increased. The components of these materials such as nicotinamide adenine dinucleotide (NAD) are capable of reducing Ag salts (silver ions) into Ag NPs. Nicotinamide adenine dinucleotide-dependent reductase can produce Ag NPs by enzymatic reduction; however, the enzymatic reduction rate is often slow (Ge, L.; Li, Q et al., 2014).

Briefly, nanoparticles are synthesized using a physical method that physically pulverizes a metal. Compared to chemical methods, thin films and the uniformity of nanoparticles distribution can be prepared with the absence of solvent contamination; however, a stable high energy over a long time should be supplied in physical methods to produce a high yield of Ag NPs of uniform size, and require large space for equipment. Evaporation/vapor condensation, arc discharge, and energy ball milling are some of the commonly used methods for synthesizing nanoparticles. Tien et al. reported the synthesis of 20–30 nm diameter of Ag NPs via arc discharge with no added surfactants. The fabrication consumes silver rods at a rate of 100 mg/min, yielding metallic silver nanoparticle and ionic silver with concentrations of approximately 11 ppm and 19 ppm, respectively. Nakamura et al. developed a simple and rapid synthesis technique (20-min irradiation), via laser irradiation of an aqueous solution of inorganic ions for nanoparticles synthesis. As a result, antibacterial calcium phosphate sub-microspheres containing Ag NPs expected to be useful in dental healthcare and infection control were produced with one-pot fabrication (Asanithi, P. et al., 2012; Iravani, S. et al., 2014; Nakamura, M. et al., 2016).

1.4 Characterization of nanoparticles:

The unique characteristics determines the potential and application of a nanoparticle. The nanoparticle characterization is carried out by various measurement techniques that is illustrated in Figure (1-3).

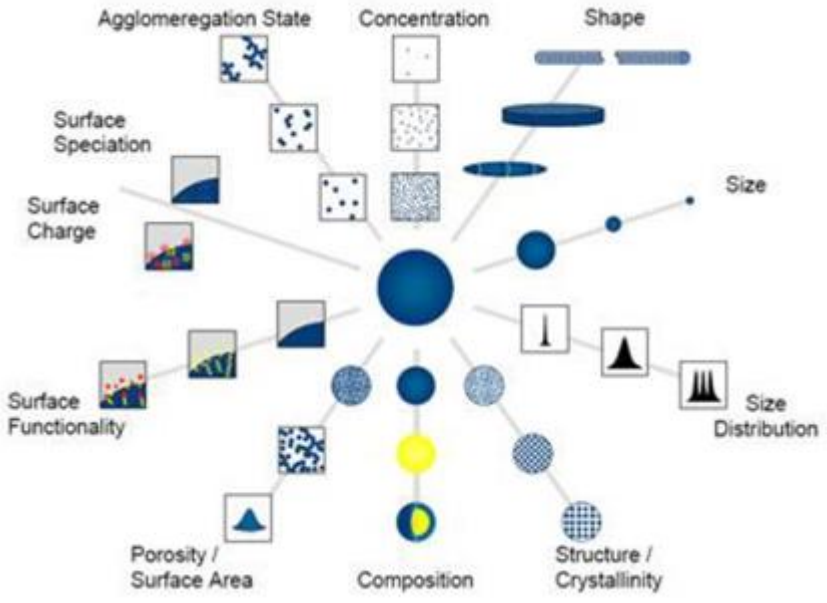

Figure (1-3): Nanoparticle characterization

1.4.1 Size:

The particle is one of the most basic and important measurement for nanoparticle characterization. It determines the size and distribution of the particle and whether it falls under nano or micro scale. The particle size and distribution are most commonly measured using electron microscopy. The images of Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) are used for the measurement of particles and clusters whereas laser diffraction methods are used for measuring bulk

samples in solid phase (Marsalek R et al., 2014). The particles in liquid phase are measured using photon correlation spectroscopy and centrifugation. The particles in gaseous phase are difficult and irreverent to use the imaging techniques and hence a Scanning Mobility Particle Sizer (SMPS) is used which provides a fast and accurate measurements compared to other methods.

1.4.2 Surface area:

The surface area is also a significant factor in nanoparticle characterization. The surface area to volume ratio of a nanoparticle has a huge influence on its performance and properties. The surface area is most commonly measured using BET analysis. A simple titration is sufficient for the surface area analysis of particles in liquid phase, but it is a labour-intensive process. Hence nuclear magnetic resonance spectroscopy (NMR) is used. A modified SMPS and differential mobility analyzer (DMA) is used for the measurement of surface are of nanoparticles in gaseous phase.

1.5 Fourier transform infrared (FTIR) spectroscopy:

Fourier transform infrared (FTIR) spectroscopy is an experimental technique used initially for qualitative and quantitative analysis of organic compounds, providing specific information on molecular structure, chemical bonding and molecular environment. For many years, FTIR has been successfully employed for studying a wide variety of proteins, enzymes, nucleic acids, lipids and glycolipids and photobiological systems. Time-resolved (tr)-FTIR spectroscopy can monitor reactions of the amino acids, the ligands and specific water molecules in the active centre of a protein in the time range from nanoseconds to seconds, thereby providing a detailed understanding of the molecular reaction mechanism.

The underlying principle of a Fourier transform infrared spectrometer is the separation of an incoming infrared light beam into two individual beams using an optical beam splitter, followed by variation of the optical path difference between these two beams using a movable mirror for one beam and a fixed mirror for the other, and by recombination of the two separate beams using an optical combiner so that interference occurs.

It can be shown that this interferogram is the Fourier transform of the original spectrum. To obtain the original spectrum from the measured interferogram, the interferogram is numerically Fourier transformed using the fast Fourier transform.

FTIR provides several advantages compared to other spectroscopic techniques: (1) simultaneous recording of spectra over a broad spectral range at any desired resolution (multiplex advantage); (2) high optical throughput because a circular aperture is used instead of a narrow slit as in conventional spectrometers (Jacquinot advantage) and (3) very accurate optical calibration by the use of a frequency-stabilised reference laser for determination of the optical path-difference between the two light beams (Connes advantage). Because of these advantages, small spectral changes can be measured accurately by FTIR using the differences between spectra recorded under specific conditions (FTIR difference spectroscopy) (Surewicz WK et al., 1993; Alben et al., 1996).

1.6 Atomic force microscopy (AFM):

Atomic force microscopy is an amazing technique that allows us to see and measure surface structure with unprecedented resolution and accuracy. An atomic force microscope (AFM) allows us, for example, to get images showing the arrangement of individual atoms in a sample, or to see the structure of individual molecules. By scanning in ultrahigh vacuum at cryogenic temperatures the hopping of individual atoms from a surface has been measured (Hoffmann, R et al., 2007). On the other hand, AFM does not need to be carried out under these extreme conditions, but can be carried out in physiological buffers at 37 8C to monitor biological reactions and even see them occur in real time (Crampton, N et al., 2007; Ando, T et al., 2007; Yokokawa, M et al., 2006). Very small images only 5 nm in size, showing only 40–50 individual atoms, can be collected to measure the crystallographic structure of materials, or images of 100 micrometres or larger can be measured, showing the shapes of dozens of living cells at the same time (Sullivan, C et al., 2005; Doktycz, M et al., 2003; Schimmel, T et al., 1999; Sugimoto, Y et al., 2007; Tromas, C et al., 2005). AFM has a

great advantage in that almost any sample can be imaged, be it very hard, such as the surface of a ceramic material, or a dispersion of metallic nanoparticles, or very soft, such as highly flexible polymers, human cells, or individual molecules of DNA. Furthermore, as well as its use as a microscope, which is to say as an imaging tool, AFM has various 'spectroscopic' modes, that measure other properties of the sample at the nanometre scale. Because of this, since its invention in the 1980s, AFM has come to be used in all fields of science, such as chemistry, biology, physics, materials science, nanotechnology, astronomy, medicine, and more. Government, academic and industrial labs all rely on AFM to deliver quantitative high-resolution images, with great flexibility in the samples that can be studied

An AFM is rather different from other microscopes, because it does not form an image by focusing light or electrons onto a surface, like an optical or electron microscope. An AFM physically 'feels' the sample's surface with a sharp probe, building up a map of the height of the sample's surface.

1.7 Applications of nanoparticles:

1. Surface coatings in biological applications: The surface of the nanoparticles should be polar to provide good aqueous solubility and prevent nanoparticle coagulation. Highly charged surfaces lead to non-specific interactions, while the polyethylene glycol terminated cells avoid non-specific bindings. Biomolecules can be attached to the nanoparticles to direct them to specific sites in the body, even specific organelles in a cell, or to monitor individual protein or RNA molecules.
2. Health and Safety: There are various speculations both medically as well as environmentally that nanoparticles are hazardous. Owing to their huge surface areas, these particles are highly reactive or catalytic. As they are extremely small, they can pass through the cell membrane and may interact with the cell organelles.
3. Nanomedicine: Nanomedicine implies the application of nanotechnology for medical uses. It includes medical uses of nanomaterials and biological devices, nanoscaled biosensors, etc. Future generation applications include biological nanoscaled machines. However, the possibilities of toxicity and environment impact of nanomaterials is an important concern.
4. Drug Delivery: One of the most celebrated application of nanotechnology is in targeted drug delivery to specific cells. Due to the possibility of transport of medicine directly to the affected area, the drug consumption can be minimised. This also lowers the side effects caused by the drugs.
5. Cancer Treatment: Owing to the large surface area to volume ratios, nanoparticles can attach multiple functional groups to it, which can locate and bind to specific tumor cells. Furthermore, the small size of nanoparticles (10- 100 nm) allows them to preferentially accumulate at tumor sites (as tumors lack an effective lymphatic drainage system).
6. Imaging: Nanoparticles have great potential as in vivo imaging tools and devices. Nanoparticle based contrast agents, images (e.g, ultrasound) can have favourable distribution and enhanced contrast. Nanoparticles can aid visualisation of various stages in cardiovascular problems such as blood pooling, angiogenesis, atherosclerosis, etc.
7. Sensing: Nanotechnology-on-a-chip is analogous to the lab-on-a-chip technology. Sensor test chips containing thousands of nanowires, able to detect proteins and other biomarkers left behind by cancer cells, could enable the detection and diagnosis of cancer in the early stages from a few drops of a patient's blood.

1.8 Applications of nanoparticles in drug delivery:

Nanotechnological application is significantly important in the field of drug delivery because of its high specificity towards the target site, so it is able to reduce toxic side effects of drugs to normal cells. The Nanoparticles (NP) plays a vital role and it can conjugate with various drugs by different methods to deliver drugs to the target site. The NP surface is designed with ligands to get affinity

towards specific cells and co-polymers to get protection from immune cells. The nanoparticles conjugated drug can ultimately recognize the site and join to the target and enter to the cell by receptor mediated endocytosis. Then NPs are able to release drugs controllably to cure diseases.

A list of some of the applications of nanoparticles in drug delivery is given below:

1. Nanoparticles for Brain Delivery.
2. Nanoparticles for Lymph Targeting.
3. Nanoparticles for Ocular Delivery.
4. Nanoparticle for Oligonucleotide Delivery.
5. Antibody targeting of nanoparticles.
6. Nanoparticles for DNA Delivery.
7. Nanoparticles for vaccine delivery.
8. Lipid nanoparticles and nanostructured lipid carriers.
9. Hydrogel nanoparticles in drug delivery.

1.9 Antimicrobial activity:

Antimicrobial activity can be defined as a collective term for all active principles (agents) that inhibit the growth of bacteria, prevent the formation of microbial colonies, and may destroy microorganisms.

Antimicrobial activity of silver, gold, copper and zinc nanoparticles have been demonstrated by some scientists across the world. When compared to chemically synthesized nanoparticles, the biogenic nanoparticles show higher antimicrobial activity. This property of green synthesized nanoparticles can be attributed to the influential action of proteins which act as capping agents. One of the promising candidates of the actinomycete family is the Streptomyces sp. which is involved in the production of antibiotics. They are capable of producing a large number of secondary metabolites. Silver nanoparticles synthesized using Streptomyces hygroscopicus were effective against bacteria such as Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Salmonella typhimurium and the pathogenic yeast, Candida albicans (Yehia E et al., 2020).

1.10 Anticancer activity of silver nanoparticles:

The silver nanoparticles proved unique anticancer activity against different types of cancer cells. The several syntheses approach significantly affect the cytotoxic activity of the achieved Ag nanoparticles.

The biological effects of various metal nanoparticles in p53- deficient tumor cells as well as in vitro tumor stroma and in vivo metastasis models were investigated (Melaiye A et al., 2004). A higher cytotoxicity was recorded for the smaller, 5 nm sized silver nanoparticles compared to their larger counterparts. Additionally, it was concluded that silver nanoparticles could induce apoptosis-dependent programmed cell death in the absence of the tumor suppressor p53. Conventional cancer therapy often fails to cause cell death in p53-deficient cancer cells. The unique chemotherapeutic potential of such developed AgNPs was proved. Moreover, it was concluded that nanoparticles of size 5-35nm primarily induced cell death through the mitochondrial structure and function targeting. Although the smaller Ag nanoparticles are more cytotoxic, the apoptotic action mechanism of both 5 and 35nm was identical (Melaiye A et al., 2005). Interestingly, the cytotoxic features of silver and silver hybrid nanoparticles are cell-type dependent. In this domain, a higher cytotoxicity was recorded against cancer cells compared to non-cancerous fibroblasts. Conclusively, the stimulation of tumor- associated fibroblast cells with metal nanoparticles represents a typical therapeutic strategy. Since the treatment by Ag and Ag hybrids suppress the cancer cell promoting the activity of a tumor associated fibroblasts. Additionally, the in vivo results proved the ability of Ag/hybrids to inhibit the

4T1 tumor metastatic spreading in mice. Impressively, Ag hybrids can enhance the therapeutic efficacy of intravenous doxorubicin treatment (Kascatan-Nebioglu A et al., 2006).

Silver nanoparticles can be an innovative approach for cancer treatment, in two perspectives: they reveal intrinsic anticancer properties, and can be used as carriers of anticancer drugs, enabling a therapeutic of dual treatment. Regarding the latter approach, the transport systems have many advantages over free anticancer substances, which were mentioned previously. In addition to these benefits already listed, there is the fact that silver itself has anticancer activity (Zhang et al., 2016; Palai et al., 2019; Pudlarz et al., 2018). For these reasons and considering that effective cancer treatment continues to be a big challenge nowadays, it is relevant to have updated knowledge about the state of the art of silver nanoparticles coupled with anticancer drugs and if it is in fact a promising approach forcancer treatment. Thus, the revision will include the obtained results with cellular assays for each case encountered in the scientific literature.

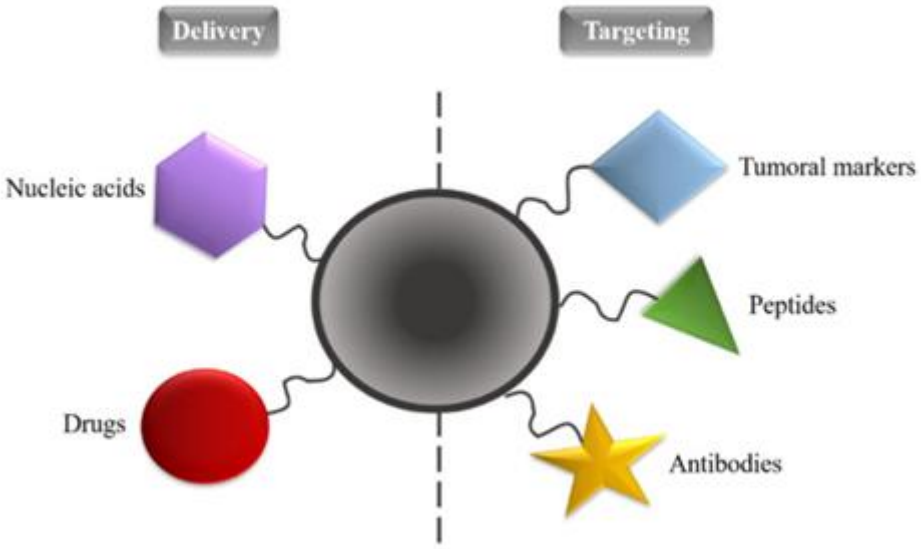

Figure (1-4): Application of noble metal nanoparticles in cancer

CHAPTER TWO

Materials and Methodes

2.1. Chemicals and instruments.

2.2. Silver nanoparticles preparation.

2.3. Silver-loaded Levofloxacin.

2.4. Evaluation of anticancer activity.

2.5. Statistical analysis.

CHAPTER TWO

Materials and Methods

2.1. Chemicals and instruments:

No.	Items	Company	Country
1	Silver nitrate	Alfa	UK
2	Filter paper	Winlab	Germany
3	Hot plate Magnetic stirrer	Four E's	China
4	Glass Erlenmeyer Flask	Pyrex	Germany
5	Ethanol	Duksan	Korea

6	Round Flask	lab max	Germany
7	Balance 4 digits	Hangzhou	China
8	Levofloxacin	TAD	Germany
9	sodium alginate polymer	Sigma-Aldrich.	USA

2.2. Silver nanoparticles preparation:

The preparation of NiO NP was performed according to the procedure described by (Balavandy et al., 2015) with minor changes. A magnetic stirrer (900 rpm) at a temperature of 60 °C. Dissolved 1.5 g of sodium alginate in 100 ml of deionized water. Then 1.5 g of silver nitrate (AgNO3) was added to the above solution with stirring for 60 min. After complete homogenization, 1 M of sodium hydroxide (NaOH) was added drop-wise until the colour turned to dark brown.

2.3. Silver-loaded Levofloxacin:

Typically, 0.6 gm of Levofloxacin was added to 50 ml water. The Livo-AgNPs nanocomposite was prepared by mixing a solution of Livo with a known weight of Ag NPs (0.1 gm/ 50 ml). The product was magnetically stirred for 18 h to facilitate Livo uptake. The nanocomposites were collected using centrifugation and then washed five times using deionized water. These were denoted as Livo-AgNPs nanocomposites.

2.4. Evaluation of anticancer activity:

2.4.1. Cell culture:

The MCF-7 and AMJ13 cancer cell lines (provided by the Iraqi Center for Cancer and Medical Genetic Research / Almustansyria University/ Iraq) were seeded in RPMI 1640 medium supplemented with 10,000 IU penicillin, 10% fetal bovine serum, and 100 µg/ml streptomycin as antibiotics in 96-well culture plates. The culturing conditions included humidified atmosphere of 5% CO2, where the plates were incubated at 37 °C in a CO2 incubator (Vijaya Kuma et al., 2009).

2.4.2. MTT cytotoxicity assay:

All the procedures of solutions preparation and the experimental tests followed the kit manufacturer's instructions (MTT Kit /Intron Biotech, Korea). 1 x 104 cells/ ml were cultured in 96-well plates and the volume was completed to 200µl with RPMI 1640 medium for each well. The plates were covered with a sterile parafilm, gently stirred, and incubated for 24 hours at 37 °C with 5% CO2. After that, the medium was removed and 200µl of Ag-levo NPs (125, 250 and 500 µg/ml) was added to the wells. in addition to other wells which contained positive control (doxorubicin 50 mg/ml) and negative control (DMSO) in each experimental repeat (Three replicates were performed for each control and concentration treatment. The plates were re-incubated for 48 hours at 37 °C with 5% CO2. After treatment with Ag-levo NPs, 10µl of MTT solution was added to each well and the plates were re-incubated for four hours at 37 °C, 5% CO2. Thereafter, 100µl of DMSO solution was added to each well after the removal of the medium and incubated for five minutes. The cell viability was estimated by measuring the optical density at a wavelength of 575 nm of absorbance and calculated according to the following formula:

Cell Viability% = Optical density of sample x 100%

Optical density of control

2.4.3. Antioxidant activity:

The antioxidant activity of the NPs was evaluated as previously described in (Vertuani et al., 2004), with minor adjustments. Utilizing stable DPPH radicals, the scavenging activities of AgNPs, and AgNPs-Levo were evaluated at 6.25, 12.5, 25, 50, and 100 microgram ml-1Seven hundred and fifty microliters of each sample were mixed with 750 UL of previously prepared DPPH solution (0.02 g DPPH in 50 mL methanol). To prepare the negative control, 750 microliters of DPPH solution was mixed with 750 microliters of methanol, while the positive control was prepared by mixing 750 microliters of DPPH with 0.5 g of ascorbic acid (vitamin C) at a concentration of 5 g mL-1. The test

samples and controls were kept at 37 C for 30 min and were protected from light before determining their optical density (OD) at 517 nm. The sample with the lowest OD showed the highest scavenging activity (%) of DPPH, which was calculated using Equation (1).

2.5. Statistical analysis:

One-way analysis of variance (ANOVA) was used in this study. The significance of the differences and the correlations among the results were evaluated using SPSS version 23. p values ≤ 0.05 were adopted to denote statistically significant differences. Data were expressed as mean ± standard deviation.

CHAPTER THREE

Results

3.1. Synthesis and characterization of Ag-Levo nanoparticles.

3.2. Characterization of nanoparticles.

3.3. Antioxidant activity.

CHAPTER THREE

Results

3.1. Synthesis and characterization of Ag-Levo nanoparticles:

The AgNPs-Livo was prepared by adding SDS to control size and stability, with tri-sodium citrate dihydrate as a reducing agent, to produce the AgNPs. One of the most frequently employed methods to prepare AgNPs is by chemical reduction, due to the method's high yield, low cost, and simplicity (Choi Y.J. et al., 2018). The loading of Livo onto the AgNPs was performed to enhance the activity of the anticancer activity changing colour from yellow to orange was an important indicator of successful NP-antibiotic conjugation.

3.2. Characterization of nanoparticles:

3.2.1. FT-IR analysis:

Measurements of FTIR were performed using infrared spectroscopy and KBr disks. The sample powder was prepared using a centrifuge for solutions at 13000 rpm for 15 minutes. The solid layer containing Silver nanoparticles and the crystalline complex was dehydrated at 37 ° C. The analysis of the products formed by FTIR measurements was performed in the range of (400_4000cm-1). FT-IR of levofloxacin showed the following characteristic peaks peak at 3265cm-1 returns to the carboxylic group, 2931cm-1 to alkanes group stretching, 1724cm-1 to stretching of Carbonyl group, 1294cm-1 to stretching of amines, 1100-1400cm-1 to the presence of halogen group Fig (3-1). While FT-IR of silver Nanoparticle shows the presence of spilt peaks of nanoparticles separately at 426.28, 727.61 cm-1, this indicates good crystalline growth, peak 1647.26 - 1631.83cm-1 is due to nitrate group NO3 Fig (3-2). The resulting complex of the silver bond (Ag+ 1) with the levofloxacin is a crystal that does not dissolve in water. This is evidence of the association of silver nanoparticles with levofloxacin chemical link by the carboxylic group of Levofloxacin by displacement citrate capped Ag-NPs, Fig (3-3) which indicates the absence of the hydroxyl-OH group and C = 0 group of Levofloxacin and its association with C-O-Ag.

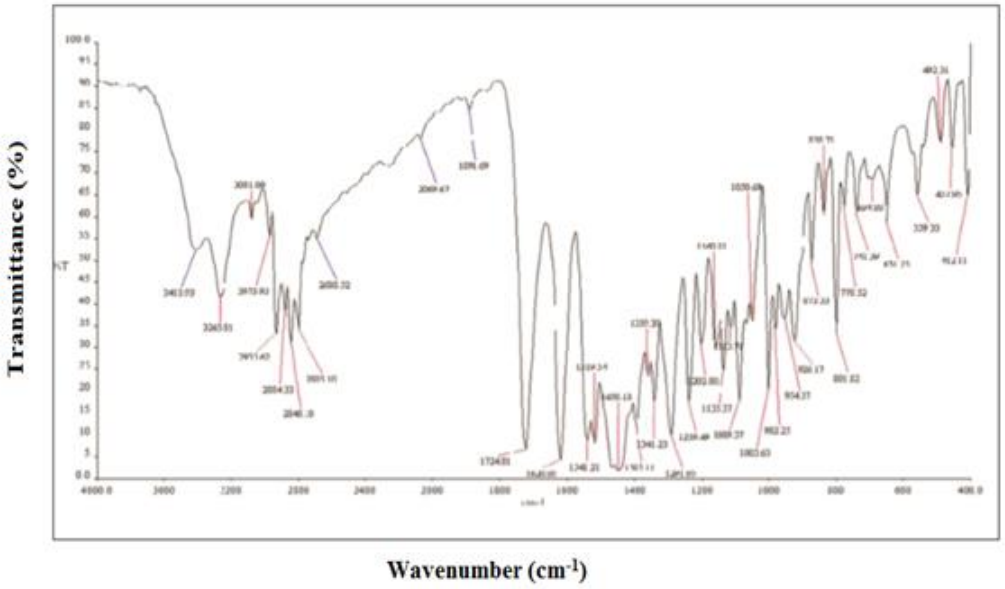

Figure (3-1): Fourier transforms infrared spectroscopy measurements of Levofloxacin NPS

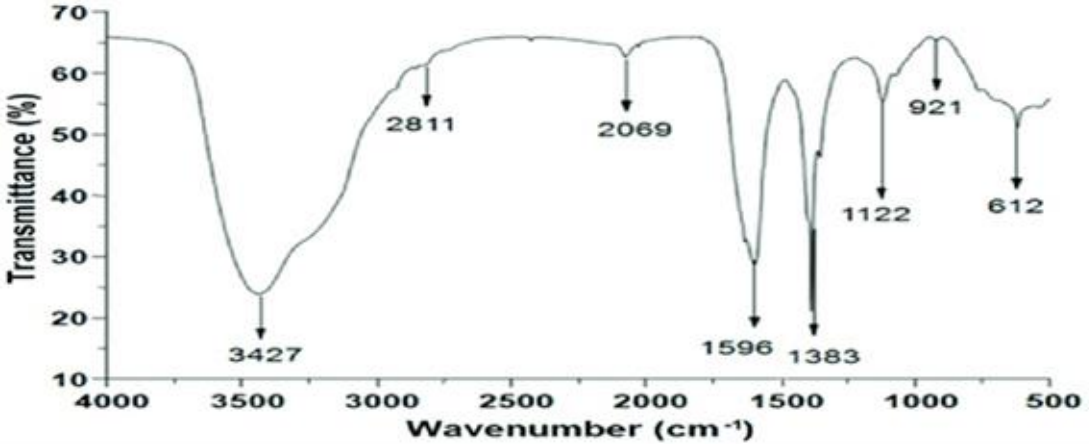

Figure (3-2): Figure (3-1): Fourier transforms infrared spectroscopy measurements of Ag NPS

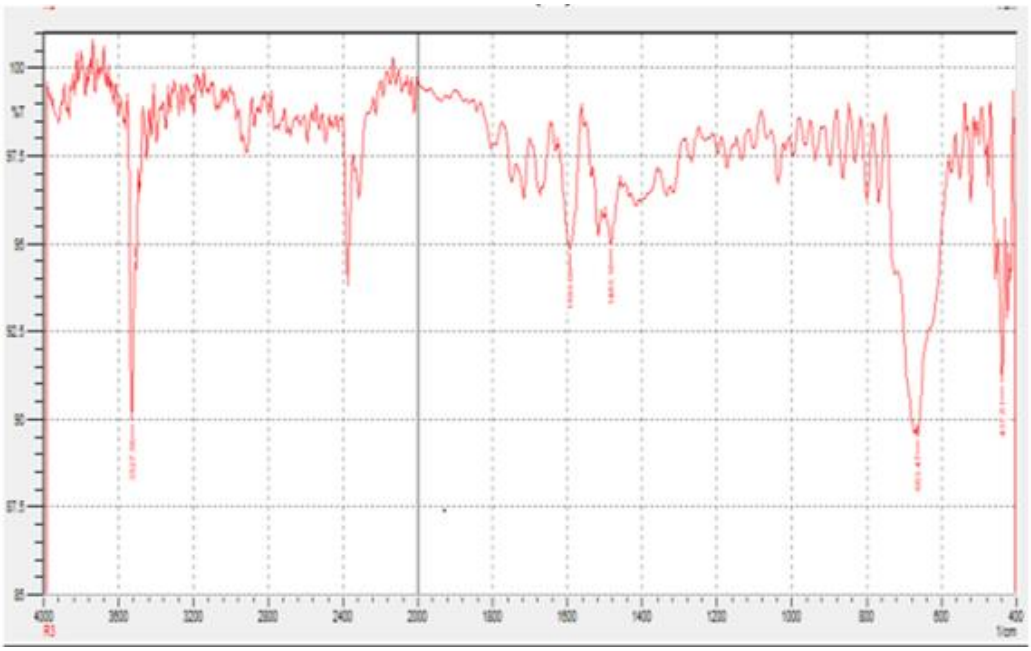

Figure (3-3): Figure (3-1): Fourier transforms infrared spectroscopy measurements of Ag-Levo NPS

3.2.2. XRD analysis:

The XRD pattern of AgNPs at angle 2_ showed four peaks at 32.12_, 38.04_, 46.21_, and 64.18_, corresponding to the (101), (111), (200), and (220) planes, respectively, when compared with the Joint Committee on Powder Diffraction Standards (JCPDS), File no. 04-0783 and 84-0713 (Figure 2). Four peaks were also detected for AgNPs-Levo at angle 2_: 32.22_, 38.04_, 44.42_, and 64.24_. These peaks corresponded to planes (101), (111), (200), and (220), respectively.

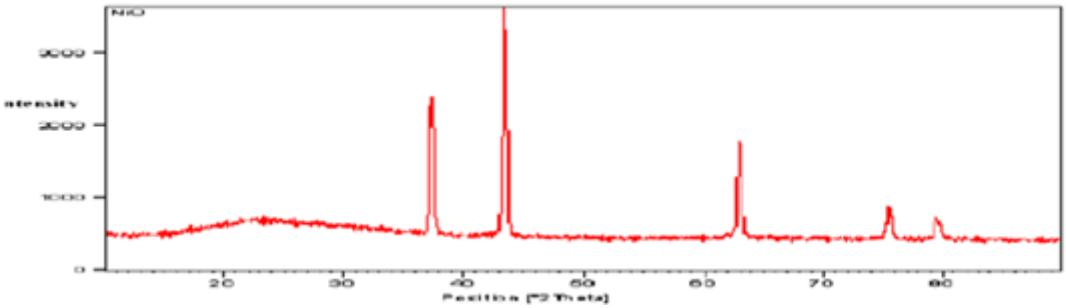

Figure (3-4): XRD analysis

The morphological features of the prepared Ag-levo NPs were investigated using SEM, as shown in Figure (3-5). The results suggest spherical shapes ranging from 10 to 40 nm in diameter. Besides, because of the heat produced in the annealing process, some of the particles were slightly agglomerated. However, the small-sized particles were very reactive because of their sharp edges, which have a high volume-to-surface ratio (higher portions of external atoms) with lower energy than that of the bulk materials.

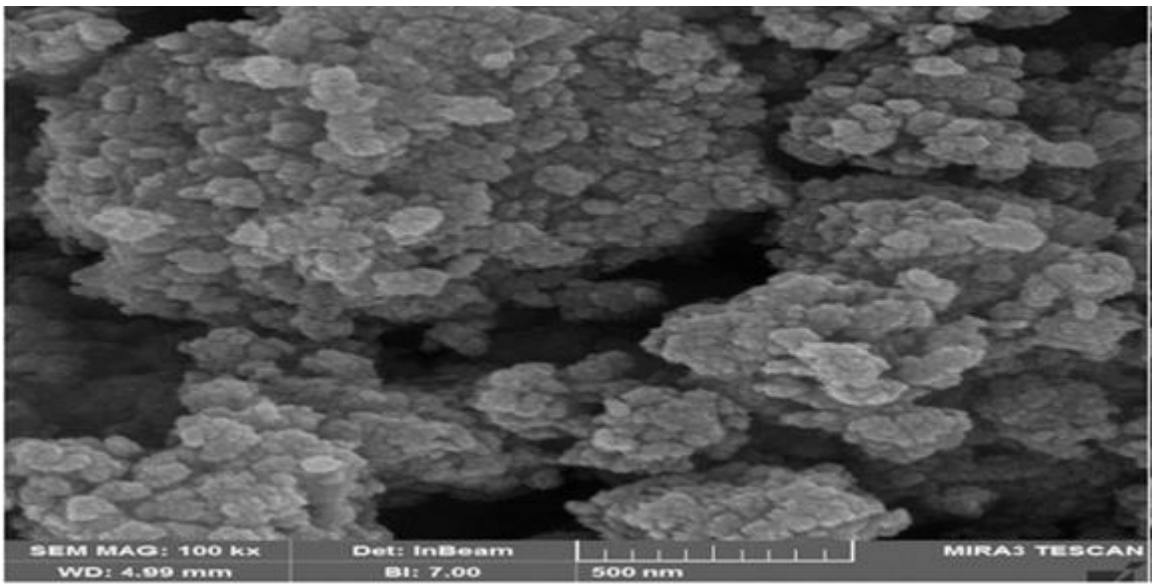

Figure (3-5): Field emission scanning electron microscopy (FE-SEM) of Ag-Levo NPs

3.3. Antioxidant activity:

DPPH, a free radical that is stable at room temperature, displays a dark violet colour when dissolved in organic solvents and shows an absorption wavelength of 517 nm. When AgNPs were present in the analysis, the DPPH stability was found to have decreased, and the violet colour turned yellow due to the presence of phenolic OH groups (Deligiannakis et al., 2012). The assays conducted for AgNPs, and AgNPs-Levo showed that DPPH was scavenged proportionally to the concentrations of the AgNPs, i.e., at concentrations of 6.50, 12.5, 25, 50, and 100 g mL□1 of AgNPs, the DPPH free radicals' scavenging capacities were 40.45%, and 73.64%, respectively Figure (3-6). The antioxidant activities of the AgNPs- and AgNPs-Levo were at 76.24% and 81.44%, respectively. The highest scavenging activity was obtained for AgNPs-Levo at 86.34%. However, this was still lower than the antioxidant activity of the ascorbic acid, the positive control standard that was used, which showed

strong antioxidant activity (Hasson et al., 2021). The antioxidant activities of the Ag NPs –Ag NPs-Levo were measured at 100 g mL 1 of concentration.

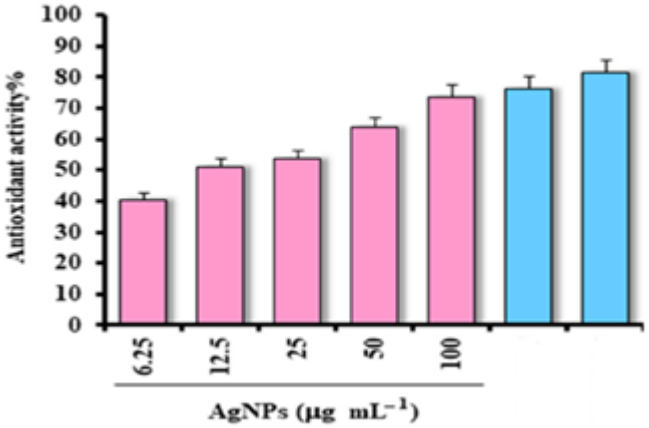

Figure (3-6): Antioxidant activity of AgNPs, AgNPs-Levo using the DPPH assay method. Ascorbic acid was used as the positive control

the results of anticancer activity were in concordance with those of antioxidant activity. Figure (3-6) shows the cytotoxicity effects of Ag-levoNPs against two cancer cell lines measured by MTT assay. Three different concentrations of NiO NPs (125,250 and 500 µg/ml) were tested and a significant ($P \leq 0.0001$) cytotoxicity effect was observed for all studied concentrations against both tested types of cancer cells in comparison with positive (DMSO) and negative (Serum-free media) controls. Cell viability was decreased up to 32% for MCF-7 and 40% for AMJ13 at the highest concentration (500 µg/ml) in comparison with DMSO and serum-free media, which reached 20% and 98%, respectively. MTT assay measures the number of viable cells to show the number of dead cells after any treatment (Marwa M et al., 2017). Evidence from previous in vitro animal models and epidemiological studies indicates that nanoparticles inhibit growth and induce apoptosis in a variety of cancer types. These characteristics of nanoparticles may be related to antioxidant, anti-mutagenic, and other biological activities (Vijaya Kuma et al., 2009).

CHAPTER FOUR

Conclusion

4.1. Conclusion.

4.2. Recommendations.

CHAPTER FOUR

Conclusion

4.1. Conclusion:

1. Ag-Levo NPs synthesized by the simple method were characterized by UV–visible spectroscopy where a final SPR band was at (366nm). The crystallinity is determined by X-ray Diffraction (XRD). The surface morphology of the Ag-Levo NPs by atomic force microscopy (AFM) gave 3D topological for Ag-Levo NPs and the size was estimated (47.52) nm.
2. Cytotoxicity of TiO2NPs by MTT assay and found minimum inhibition concentration at (12.5) g/ml for WRL-68 and TCP-1013.

4.2. Recommendations:

1. Possibility of synthesized NPs with different ion metals such as Zn, Cu, and Fe.
2. Possibility of synthesizing Ag NPs by using other extracts (plant, bacteria, fungi)
3. Study the parameter of reaction time in the optimization of synthesis Ag-Levo NPs.

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