“SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES LOADED MICROSPONGES”

A PROJECT

Submitted in partial fulfillment of the requirements for the award of the degree of

BACHELOR OF PHARMACY

Under The Supervision of

Dr. GOPAL SINGH BISHT

BY

SHIVANSH SHRIVASTAVA (121752)

AASHISH KAUNDAL (121757)

JAYPEE UNIVERSITY OF INFORMATION AND TECHNOLOGY

WAKNAGHAT SOLAN – 173 234

HIMACHAL PRADESH, INDIA

CERTIFICATE

This is to certify that the work which is being presented in the project entitled —"Synthesis and Characterization of Silver Nanoparticles Loaded Microsponges" in partial fulfillment of the requirements for the award of the degree of Bachelor of Pharmacy and submitted in Pharmacy Department, Jaypee University of Information Technology, Waknaghat is an authentic record of work carried out by Shivansh Shrivastava and Aashish Kaundal during a period from August 2015 to May 2016 under the supervision of Dr. Gopal Singh Bisht, Assistant Professor, Department of Bioinformatics and Biotechnology, Jaypee University of Information Technology, Waknaghat.

The above statement made is correct to the best of my knowledge.

Signature of supervisor ………………..

Name of supervisor - Dr. Gopal Singh Bisht

Designation - Assistant Professor (Senior grade)

Date

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Thanking you,

Shivansh Shrivastava (121752) Aashish kaundal (121757)

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SUMMARY

Silver Nanoparticles are nano sized particles of silver between 1 and 100 nm. They have unique properties which help in molecular diagnostics, therapies and in designing medical devices that are used in medical procedures. Silver nanoparticle can be prepared by chemical, physical and biological methods, the major biological method involved are via bacteria, fungi, and plant extracts. Silver nanoparticle has both antimicrobial and anti-inflammatory property. In spite of these interesting properties, not much success has been achieved because of their nano-toxicity. One of the approach to deal with this toxicity is to incorporate silver nanoparticles in microsponges; as it helps in controlled or sustained release of nanoparticles thus reducing toxicity. This report provides a comprehensive view on synthesis, formulation, applications of silver nanoparticle. The focus is on efficient synthesis and controlled release of silver nanoparticles through silver nanoparticle loaded microsponges to reduce toxicity.

INTRODUCTION

Nanotechnology applications are highly suitable for biological molecules, because of their exclusive properties .The field of nanotechnology is one of the upcoming areas of research in the modern field of material science. Nanoparticle show completely new or improved properties, such as size, distribution and morphology of the particles etc. Good applications of nanoparticles and nanomaterials are emerging rapidly on various fields [3].

Metal nanoparticles due to small size have high specific surface area and a high number of surface atoms. They have unique physicochemical characteristics of nanoparticles, including catalytic activity, optical properties, electronic properties, antibacterial properties, and magnetic properties [4-7] they are gaining the interest for their research in the field of drug formulation. Over the past few years, the synthesis of metal nanoparticles is an important topic of research in modern material science. There are huge amount of application of silver crystalline nanoparticle like high sensitivity biomolecular detection, diagnostics, antimicrobials, therapeutics, catalysis and micro-electronics. However, there is need to discover more economic and lean synthesis route to synthesize the silver nanoparticles. Silver is well known for having an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes [8]. In medicines, silver and silver nanoparticles have some application including skin ointments and creams containing silver to prevent infection of burns and open wounds [9], implants and medical equipment's prepared with silver-impregnated polymers [10]. In textile industry, sport equipment are made of silver-embedded fabrics [11]. Nanoparticles can be synthesized using different approaches including chemical, physical, and biological. Chemical method for synthesizing nanoparticles requires only short period of time for large quantity of nanoparticles to prepare. This method requires capping agents for size stabilization of the nanoparticles. Chemicals used for nanoparticles synthesis and stabilization are toxic and lead to non-ecofriendly byproducts. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as byproducts. Thus, there is an increasing demand for "green nanotechnology" [12]. Many biological approaches for both extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria, fungi and plants [13, 14]. Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as provide natural capping agents. Moreover, use of plant extracts also reduces the cost of microorganism's isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms [12].

With respect to the microbes, the silver nanoparticles get attached to the cell wall, thereby disturbing the permeability of cell wall and cellular respiration. The nanoparticles may also penetrate deep inside the cell wall, thus causing cellular damage by interacting with phosphorus and sulfur containing compounds, such as DNA and protein, present inside the cell. The bactericidal properties of silver nanoparticles are due to the release of silver ions from the particles, which confers the antimicrobial activity [24]. Besides, the potency of the antibacterial effects corresponds to the size of the nanoparticle. The smaller particles have higher antibacterial activities due to the equivalent silver mass content. With respect to the clinical applications of nanoparticle, microorganisms including diatoms, fungi, bacteria and yeast producing inorganic materials through biological synthesis either intra or extracellularly made nanoparticles more biocompatible [25].

Silver nanoparticle synthesized by any route may cause some toxicity when administered .This is due to increased concentration of silver ions in tisues. This can be minimized by formulating silver nanoparticle in a controlled release formulation .Microsponges are polymeric delivery systems composed of porous microspheres. They are tiny sponge-like spherical particles with a large porous surface. Moreover, they may enhance stability, reduce side effects and modify drug release favorably. Microsponge technology has many favorable characteristics, which make it a versatile drug delivery vehicle. Microsponge systems are based on microscopic, polymer-based microspheres that can suspend or entrap a wide variety of substances, and can then be incorporated into a formulated product such as a gel, cream, liquid or powder. The outer surface is typically porous, allowing a sustained flow of substances out of the sphere. Microsponges are porous, polymeric microspheres that are used mostly for topical use and have recently been used for oral administration. Microsponges are designed to deliver a pharmaceutical active ingredient efficiently at the minimum dose and also to enhance stability, reduce side effects, and modify drug release.

LITERATURE REVIEW

Nanotechnology is an important field of modern research dealing with design, synthesis, and manipulation of particles structure ranging from approximately 1-1000 nm in one dimension. Remarkable growth in this up-and-coming technology has opened novel fundamental and applied, including the synthesis of nanoscale materials and exploration or utilization of their exotic physicochemical properties. Nanotechnology is rapidly gaining importance in a number of areas such as health care, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics, space industries, drug-gene delivery, and photo electrochemical applications [26, 27]. Nanomaterials in the field of medical like medicine like anti-bacterial and anti-fungal agent, and water treatment. In the context of global efforts to reduce drug resistance caused by antibiotics, silver nanoparticles can be used.

Nanotechnology is fundamentally changing the way in which materials are synthesized and devices are fabricated. Incorporation of nanoscale building blocks into functional assemblies and further into multifunctional devices can be achieved through a "bottom-up approach" Research on the synthesis of Nano sized material is of great interest because of their unique properties like optoelectronic, magnetic, and mechanical, which differs from bulk [28].

NANOPARTICLES:

The term "nanoparticles" is used to describe a particle with size in the range of 1nm-1000 nm, at least in one of the three possible dimensions. In this size range, the physical, chemical and biological properties of the nanoparticles changes in fundamental ways from the properties of both individual atoms/molecules and of the corresponding bulk materials. Nanoparticles can be made of materials of diverse chemical nature, the most common being metals, metal oxides, silicates, non-oxide ceramics, polymers, organics, carbon and biomolecules. Nanoparticles exist in several different morphologies such as spheres, cylinders, platelets, tubes etc. Generally the nanoparticles are designed with surface modifications tailored to meet the needs of specific applications they are going to be used for. The enormous diversity of the nanoparticles arising from their wide chemical nature, shape and morphologies, the medium in which the particles are present, the state of dispersion of the particles and most importantly, the numerous possible surface modifications the nanoparticles can be subjected to make this an important active field of science now-a-days.

SILVER NANOPARTICLES:

Silver nanoparticles have unique properties (e.g., size and shape depending optical, electrical, and magnetic properties) which can be incorporated into different antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components. Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles [30, 31]. The most popular chemical approaches, including chemical reduction using a variety of organic and inorganic reducing agents, electrochemical techniques, physicochemical reduction, and radiolysis are widely used for the synthesis of silver nanoparticles. Recently, nanoparticle synthesis is among the most interesting scientific areas of inquiry, and there is growing attention to produce nanoparticles using environmentally friendly methods (green chemistry). Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, Tollens, biological, and irradiation method which have advantages over conventional methods involving chemical agents associated with environmental toxicity. This chapter presents an overview of silver nanoparticle preparation by physical, chemical, and green synthesis approaches. The aim of this chapter is, therefore, to reflect on the current state and future prospects, especially the potentials and limitations of the above mentioned techniques for industries. Moreover, we discuss the applications of silver nanoparticles and their incorporation into other materials, the mechanistic aspects of the antimicrobial effects of silver nanoparticles.

METHODS FOR NANOPARTICLE SYNTHESIS:

Physical approaches:

Most important physical approaches include evaporation-condensation and laser ablation. Various metal nanoparticles such as silver, gold, lead sulfide, cadmium sulfide, and fullerene have previously been synthesized using the evaporation-condensation method. The absence of solvent contamination in the prepared thin films and the uniformity of nanoparticles distribution are the advantages of physical approaches in comparison with chemical processes. [32, 33]. It was demonstrated that silver nanoparticles could be synthesized via a small ceramic heater with a local heating source [34]. The evaporated vapor can cool at a suitable rapid rate, because the temperature gradient in the vicinity of the heater surface is very steep in comparison with that of a tube furnace. This makes possible the formation of small nanoparticles in high concentration. This physical method can be useful as a nanoparticle generator for long-term experiments for inhalation toxicity studies, and as a calibration device for nanoparticle measurement equipment [34]. Silver nanoparticles could be synthesized by laser ablation of metallic bulk materials in solution [35-39]. The ablation efficiency and the characteristics of produced nanosilver particles depend upon many factors such as the wavelength of the laser impinging the metallic target, the duration of the laser pulses, the ablation time duration and the effective liquid medium, with or without the presence of surfactants [40-43]. One important advantage of laser ablation technique compared to other methods for production of metal colloids is the absence of chemical reagents in solutions. Therefore, pure and uncontaminated metal colloids for further applications can be prepared by this technique [44].

Chemical approaches:

The most common approach for synthesis of silver nanoparticles is chemical reduction by organic and inorganic reducing agents. In general, different reducing agents such as sodium citrate, ascorbate, sodium borohydride (NaBH4), elemental hydrogen, polyol process, Tollens reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block copolymers are used for reduction of silver ions (Ag+) in aqueous or non-aqueous solutions. The aforementioned reducing agents reduce silver ions (Ag+) and lead to the formation of metallic silver (Ag0), which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to formation of metallic colloidal silver particles [46-48]. It is important to use protective agents to stabilize dispersive nanoparticles during the course of metal nanoparticle preparation, and protect the nanoparticles that can be absorbed on or bind onto nanoparticle surfaces, avoiding their agglomeration [49]. The presence of surfactants comprising functionalities (e.g., thiols, amines, acids, and alcohols) for interactions with particle surfaces can stabilize particle growth, and protect particles from sedimentation, agglomeration, or losing their surface properties. Recently, a simple one-step process, Tollens method, has been used for the synthesis of silver nanoparticles with a controlled size. In the modified Tollens procedure, silver ions are reduced by saccharides in the presence of ammonia, yielding silver nanoparticle films (50-200 nm), silver hydrosols (20-50 nm) and silver nanoparticles of different shapes [52].

Biological approaches:

In recent years, the development of efficient green chemistry methods employing natural reducing, capping, and stabilizing agents to prepare silver nanoparticles with desired morphology and size have become a major focus of researchers. Biological methods can be used to synthesize silver nanoparticles without the use of any harsh, toxic and expensive chemical substances [53, 54]. The bioreduction of metal ions by combinations of biomolecules found in the extracts of certain organisms (e.g., enzymes/proteins, amino acids, polysaccharides, and vitamins) is environmentally benign, yet chemically complex. Many studies have reported successful synthesis of silver nanoparticle using organisms (microorganisms and biological systems) [52, 53].

NANOSILVER:

One of the substances used in Nano formulation is silver (Nano silver). Due to its antimicrobial properties, silver has also been incorporated in filters to purify drinking water and clean swimming pool water. To generate nanosilver, metallic silver has been engineered into ultrafine particles by several methods; include spark discharging, electrochemical reduction, solution irradiation and cryo-chemical synthesis [47]. Nano-silver particles are mostly smaller than 100 nm and consist of about 20-15,000 silver atoms [47]. In addition, nanostructures can be produced as tubes, wires and films. At the nano-scale, the silver particles exhibit deviating physico-chemical properties (like pH dependent partitioning to solid and dissolved particulate matters) and biological activities compared with the regular metal [48].This is due to the higher surface area per mass, allowing a larger amount of atoms to interact with their surroundings. Due to the properties of silver at the nanoscale, nanosilver is nowadays used in an increasing number of consumer and medical products. Because, silver is a soft white lustrous element, an important use of silver nanoparticles is to give products a silver finish. Still, the remarkably strong antimicrobial activity is the major direction for development of nano-silver products. Examples are food packaging materials and food supplements, odour-resistant textiles, electronics, household appliances, cosmetics and medical advices, water disinfectants and room sprays.

WHY SILVER?

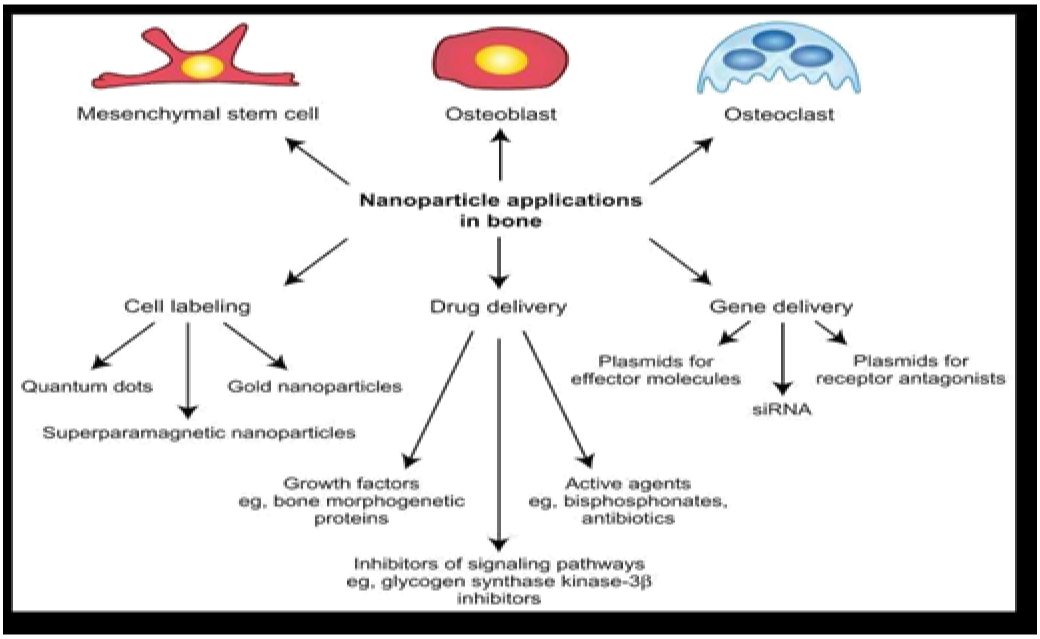
Silver is one of the basic element that makes up our planet. It is a rare, but naturally occurring element, slightly harder than gold and very ductile and malleable. Pure silver has the highest electrical and thermal conductivity of all metals and has the lowest contact resistance. Silver can be present in four different oxidation states: Ag0, Ag^{2+}, Ag^{3+}. The former two are the most abundant ones, the latter are unstable in the aquatic environment [49]. Metallic silver itself is insoluble in water, but metallic salts such as $AgNO_3$ and Silver chloride are soluble in water (WHO).Metallic silver is used for the surgical prosthesis and splints, fungicides and coinage. Soluble silver compounds such as silver slats, have been used in treating mental illness, epilepsy, nicotine addition, gastroenteritis and infectious diseases including syphilis and gonorrhea [49]. Although acute toxicity of silver in the environment is dependent on the availability of free silver ions, investigations have shown that these concentrations of Ag^+ ions are too low to lead toxicity (WHO, 2002). Metallic silver appears to pose minimal risk to health, whereas soluble silver compounds are more readily absorbed and have the potential to produce adverse effects [50]. The wide variety of uses of silver allows exposure through various routes of entry into the body. Ingestion is the primary route for entry for silver compounds and colloidal silver proteins. Dietary intake of silver is estimated at 70-90µg/day. Since silver in any form is not thought to be toxic to the immune , cardiovascular, nervous or reproductive system and it is not considered to be carcinogenic [51], therefore silver is relatively non-toxic [52]. Silver demand will likely to rise as silver find new uses, particularly in textiles, plastics and medical industries, changing the pattern of silver emission as these technologies and products diffuse through the global economy [53].

ACTION OF SILVER NANOPARTICLES ON MICROBES:

The exact mechanism which silver nanoparticles employ to cause antimicrobial effect is not clearly known and is a debated topic. There are however various theories on the action of silver nanoparticles on microbes to cause the microbicidal effect. Silver nanoparticles have the ability to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. There is formation of "pits" on the cell surface, and there is accumulation of the nanoparticles on the cell surface [53]. The formation of free radicals by the silver nanoparticles may be considered to be another mechanism by which the cells die. There have been electron spin resonance spectroscopy studies that suggested that there is formation of free radicals by the silver nanoparticles when in contact with the bacteria, and these free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death [53]. It has also been proposed that there can be release of silver ions by the nanoparticles [46], and these ions can interact with the thiol groups of many vital enzymes and inactivate them [47]. The bacterial cells in contact with silver take in silver ions, which inhibit several functions in the cell and damage the cells. Then, there is the generation of reactive oxygen species, which are produced possibly through the inhibition of a respiratory enzyme by silver ions and attack the cell itself. Silver is a soft acid, and there is a natural tendency of an acid to react with a base, in this case, a soft acid to react with a soft base. The cells are majorly made up of sulfur and phosphorus which are soft bases. The action of these nanoparticles on the cell can cause the reaction to take place and subsequently lead to cell death. Another fact is that the DNA has sulfur and phosphorus as its major components; the nanoparticles can act on these soft bases and destroy the DNA which would definitely lead to cell death [48]. The interaction of the silver nanoparticles with the sulfur and phosphorus of the DNA can lead to problems in the DNA replication of the bacteria and thus terminate the microbes. It has also been found that the nanoparticles can modulate the signal transduction in bacteria. It is a well-established fact that phosphorylation of protein substrates in bacteria influences bacterial signal transduction. Dephosphorylation is noted only in the tyrosine residues of gram-negative bacteria. The phosphotyrosine profile of bacterial peptides is altered by the nanoparticles. It was found that the nanoparticles dephosphorylate the peptide substrates on tyrosine residues, which leads to signal transduction inhibition and thus the stoppage of growth. It is however necessary to understand that further research is required on the topic to thoroughly establish the claims [49].

APPLICATIONS OF SILVER NANOPARTICLES AND THEIR INCORPORATION INTO OTHER MATERIALS

Nanoparticles used due to their extremely small size and large surface to volume ratio, which lead to both chemical and physical differences in their properties compared to bulk of the same chemical composition, such as mechanical, biological and sterical properties, catalytic activity, thermal and electrical conductivity, optical absorption and melting point [40]. Thus, production and designing of materials with novel applications can be resulted by controlling shape and size at nanometer scale. Nanoparticles exhibit size and shape-dependent properties which are of interest for applications ranging from biosensing and catalysts to optics, antimicrobial activity, computer transistors, electrometers, chemical sensors, and wireless electronic logic and memory schemes. Those particles also have many applications in different fields such as medical imaging, nano-composites, filters, drug delivery, and hyperthermia of tumors [51, 52]. Silver nanoparticles have drawn the attention of researchers because of their extensive applications in areas such as integrated circuits [22, 53], sensors, biolabelling ,filters , antimicrobial deodorant fibres, cell electrodes, low-cost paper batteries (silver nano-wires) and antimicrobials .Silver nanoparticles have been used extensively as antimicrobial agents in health industry, food storage, textile coatings and a number of environmental applications. Antimicrobial properties of silver nanoparticles caused the use of these nano-metals in different fields of medicine, various industries, animal husbandry, packaging, accessories, cosmetics, health and military. [47,48]. For instance, it was shown that silver nanoparticles mainly in the range of 1-10 nm attached to the surface of E. coli cell membrane, and disturbed its proper function such as respiration and permeability.



Overview of nanoparticle applications

In general, therapeutic effects of silver particles (in suspension form) depend on important aspects, including particle size (surface area and energy), particle shape (catalytic activity), particle concentration (therapeutic index) and particle charge (oligodynamic quality) [19]. Mechanisms of antimicrobial effects of silver nanoparticles are still not fully understood, but several studies have revealed that silver nanoparticles may attach to the negatively charged bacterial cell wall and rupture it, which leads to denaturation of protein and finally cell death. Fluorescent bacteria were used to study antibacterial effects of silver nanoparticles. Green fluorescent proteins were adapted to these investigations [34]. It was found that silver nanoparticles attached to sulfur-containing proteins of bacteria, and caused death. Moreover, fluorescent measurements of cell-free supernatants showed the effect of silver nanoparticles on recombination of bacteria. The attachment of silver ions or nanoparticles to the cell wall caused accumulation of envelope protein precursors resulting in immediate dissipation of the proton motive force [33]. Catalytic mechanism of silver nanoparticle composites and their damage to the cell by interaction with phosphorous- and sulfur-containing compounds such as DNA have been also investigated [10]. Furthermore, silver nanoparticles exhibited destabilization of the outer membrane and rupture of the plasma membrane, thereby causing depletion of intracellular ATP [48]. Another mechanism involves the association of silver with oxygen and its reaction with sulfhydryl groups on the cell wall to form R-S-S-R bonds, thereby blocking respiration and causing cell death [51].

Applications of silver nanoparticles in pharmaceutics, medicine, and dentistry Pharmaceutics & Medicines

1. Treatment of dermatitis; inhibition of HIV-1 replication
2. Treatment of ulcerative colitis & acne
3. Antimicrobial effects against infectious organisms
4. Remote laser light-induced opening of microcapsules
5. Silver/dendrimer nanocomposite for cell labeling
6. Molecular imaging of cancer cells
7. Enhanced Raman Scattering (SERS) spectroscopy
8. Detection of viral structures (SERS & Silver nanorods)
9. Coating of hospital textile (surgical gowns, face mask)
10. Additive in bone cement
11. Implantable material using clay-layers with starch-stabilized Ag NPs
12. Hydrogel for wound dressing
13. Dentistry
14. Additive in polymerizable dental materials Patent
15. Polyethylene tubes filled fibrin sponge embedded with Ag NPs dispersion.

Mechanisms of antibacterial effects of silver nanoparticles

1) Cell death due to uncoupling of oxidative phosphorylation
2) Cell death due to induction of free radical formation
3) Interference with respiratory chain at Cyt C level
4) Interference with components of microbial ETS
5) Interaction with phosphorous- and sulfur-containing compounds such as DNA

TOXICITY OF SILVER NANOPARTICLES:

The unique physical and chemical properties of silver nanoparticles make them excellent candidates for a number of day-to-day activities, and also the antimicrobial and anti-inflammatory properties make them excellent candidates for many purposes in the medical field. However, there are studies and reports that suggest that nanosilver can allegedly cause adverse effects on humans as well as the environment.

It is estimated that tonnes of silver are released into the environment from industrial wastes, and it is believed that the toxicity of silver in the environment is majorly due to free silver ions in the aqueous phase. The adverse effects of these free silver ions on humans and all living beings include permanent bluish-gray discoloration of the skin (argyria) or the eyes (argyrosis), and exposure to soluble silver compounds may produce toxic effects like liver and kidney damage; eye, skin, respiratory, and intestinal tract irritations; and untoward changes in blood cells [51]. In twenty-first century, nanosilver has been gaining popularity and is now being used in almost every field, most importantly the medical field. However, there have been reports of how nanosilver cannot discriminate between different strains of bacteria and can hence destroy microbes beneficial to the ecology [50].

There are only very few studies conducted to assess the toxicity of nanosilver. In one study, in vitro toxicity assay of silver nanoparticles in rat liver cells has shown that even low-level exposure to silver nanoparticles resulted in oxidative stress and impaired mitochondrial function [50]. Silver nanoparticles also proved to be toxic to in vitro mouse germ line stem cells as they impaired mitochondrial function and caused leakage through the cell membranes. Nanosilver aggregates are said to be more cytotoxic than asbestos [51]. There is evidence that shows that silver ions cause changes in the permeability of the cell membrane to potassium and sodium ions at concentrations that do not even limit sodium, potassium, ATP, or mitochondrial activity [35]. The literature also proves that nanosilver can induce toxic effects on the proliferation and cytokine expression by peripheral blood mononuclear cells [36]. Nanosilver is also known to show severe toxic effects on the male reproductive system. Research shows that nanosilver can cross the blood-testes barrier and be deposited in the testes where they adversely affect the sperm cells [37]. Even commercially available silver-based dressings have been proved to have cytotoxic effects on various experimental models [38]. In vivo studies on the oral toxicity of nanosilver on rats have indicated that the target organ in mouse for the nanosilver was the liver. It was also found from histopathological studies that there was a higher incidence of bile duct hyperplasia, with or without necrosis, fibrosis, and pigmentation in the study animals [39].

Microsponges

Microsponges are polymeric delivery systems composed of porous microspheres. They are tiny sponge-like spherical particles with a large porous surface. Moreover, they may enhance stability, reduce side effects and modify drug release favorably. Microsponge technology has many favorable characteristics, which make it a versatile drug delivery vehicle. Microsponge Systems are based on microscopic, polymer-based microspheres that can suspend or entrap a wide variety of substances, and can then be incorporated into a formulated product such as a gel, cream, liquid or powder. The outer surface is typically porous, allowing a sustained flow of substances out of the sphere. Microsponges are porous, polymeric microspheres that are used mostly for topical use and have recently been used for oral administration. Microsponges are designed to deliver a pharmaceutical active ingredient efficiently at the minimum dose and also to enhance stability, reduce side effects, and modify drug release.

Methods of Preparation of Microsponges

Liquid–liquid suspension polymerization

In general, a solution is made comprising of monomers and the functional or active ingredients, which are immiscible with water. This phase is then suspended with agitation in an aqueous phase, usually containing additives, such as surfactants and dispersants, to promote suspension. Once the suspension is established with discrete droplets of the desired size, polymerization is effected by activating the monomers either by catalysis, increased temperature or irradiation. As the polymerization process continues, a spherical structure is produced containing thousands of microsponges bunched together like grapes, forming interconnecting reservoirs.

Once the polymerization is complete the solid particles that result from the process are recovered from the suspension. The particles are then washed and processed until they are substantially ready for use. The microsponge products can be made using styrene and divinylbenzene or methyl methacrylate and ethylene glycol dimethacrylate as starting materials.[5]

Quasi-emulsion solvent diffusion

The inner organic phase, Eudragit RS 100 is dissolved in ethyl alcohol. Next, the drug is added to the solution and dissolved under ultrasonication at 35°C. The inner phase is poured into the polyvinyl alcohol solution in water (outer phase). Following 60 minutes of stirring, the mixture is filtered, to separate the microsponges. The microsponges are dried in an air-heated oven at 40°C for 12 hours (53).

Microsponges are patented polymeric delivery systems consisting of porous microspheres that can entrap a wide range of active ingredients such as emollients, fragrances, essential oils, sunscreens, and anti-infective, anti-fungal, and anti-inflammatory agents [3]. Like a true sponge, each microsphere consists of a myriad of interconnecting voids within a non-collapsible structure, with a large porous surface

MATERIAL AND METHOD

Silver nanoparticles were synthesized chemically using trisodium citrate as reducing or capping agent.

SYNTHESIS OF SILVER NANOPARTICLES USING TRISODIUM CITRATE

The reaction was carried out at the room temperature in presence of tri sodium citrate as a capping agent and silver nitrate (53).

a) 0.01M solution of silver nitrate 100 ml was prepared and boiled.
b) 1% w/v trisodium citrate in 10 ml was prepared.
c) It was added drop wise to silver nitrate solution till the color of solution becomes pale yellow.
d) Solution was centrifuged for 15 minutes at 10000 rpm.
e) Supernatant containing free silver ions and reducing agent was thrown.
f) Pellet formed at the bottom was re-suspended in small quantity of distilled water.

Preparation of microsponges

a) 0.5 gram of ethyl cellulose was weighed carefully and added to 10 ml of ethanol (Solution A).
b) 40 mg of poly vinyl alcohol was weighed and dissolved in 200 ml of distilled water

(Solution B).

c) Solution A was added dropwise to solution B with continuous homogenization for 1 hour.
d) The solution was then ultrasonicated for 35 minutes at 35 degree Celsius.
e) Solution was filtered using Whatman filter paper to separate microsponges.
f) These were collected and dried in oven at 50 degree Celsius.
g) Stored in vacuum desiccator.

Incorporation of silver nanoparticles in microsponges

a) Dried Microsponges were added to Silver nanoparticles and volume was made upto 10 ml with distilled water with continuous stirring overnight. Incorporation of silver nanoparticles into microsponges was confirmed by change in color of microsponges from white to pale yellow, as the color of silver nanoparticles was pale yellow.
b) The mixture was centrifuged for 15 minutes and supernatant containing free silver nanoparticles was thrown.
c) Pellet was collected and re-suspended in water; volume was made upto 10 ml with distilled water.

Evaluation parameters

Evaluation parameters determined were size, zeta potential, shape, percent drug loading and percent yield.

For silver nanoparticles

A) **UV-Vis Analysis**:

The optical property of silver nanoparticles was determined by UV-Vis spectrophotometer.

B**) Zeta potential and size distribution analysis of nanoparticles**

Size distribution and zeta potential analysis of formulation were done by recording intensity using (Zeta sizer).

For microsponges

A) **Microscopic analysis-**

Microscopic analysis of prepared microsponges was done using phase contrast microscope.

B**) Zeta potential and size distribution analysis of nanoparticles**

Size distribution and zeta potential analysis of formulation were done by recording intensity using (Zeta sizer).

Evaluation of silver nanoparticles incorporated in microsponges

A) **Percent loading**

Percentage of silver nanoparticles loaded into microsponges was determined by adding 1ml of silver nanoparticle-microsponge formulation and diluting it with 1ml of ethanol. Solution was filtered using 0.2 micron filter. Absorbance was recorded using UV-spectrophotometer at 370 nm. Actual concentration of drug was calculated by slight modification in method as described in literature and % drug loading was calculated using the following formula.

$$\% \text{ loading (w/v)} = \frac{\text{Amount of silver nanoparticles present in microsponge}}{\text{Amount of formulation taken}} \times 100$$

B) **Percent yield**

Percentage yield was calculated using following formula

$$\% \text{ yield} = \frac{\text{Amount of nanoparticles obtained}}{\text{Amount of snps- polymer mixture taken}} \times 100$$

RESULT

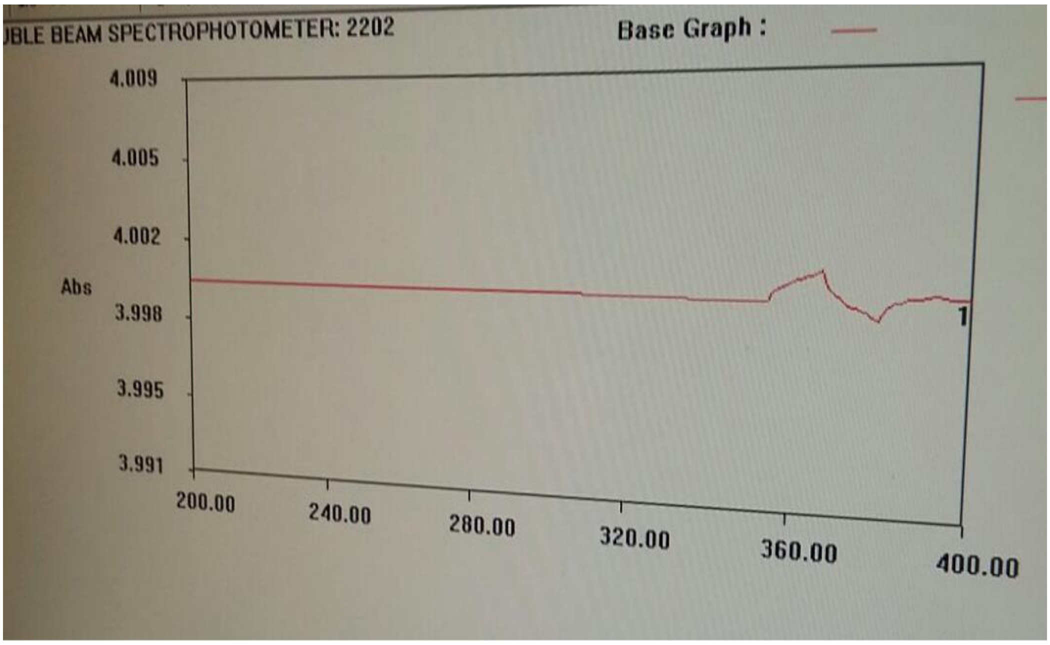
Evaluation parameters

Evaluation parameters determined were size, zeta potential, shape, percent drug loading, percent yield.

For silver nanoparticles

A) UV-Vis Analysis:

Uv-vis analysis of nanoparticles prepared from trisodium citrate. The optical property of silver nanoparticles was determined. λ max of prepared nanoparticles was found to be 370nm. Results are shown below.



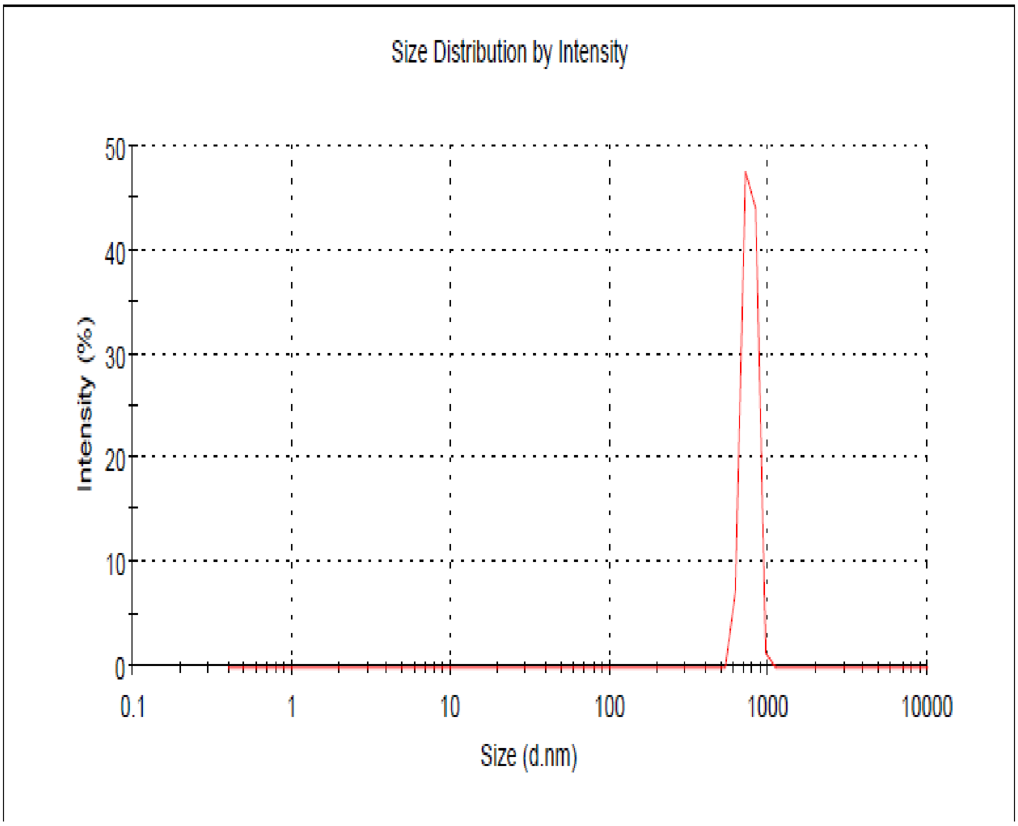
B) Zeta potential and size distribution analysis of nanoparticles

Size distribution and zeta potential analysis of formulation were done by recording intensity using (Zeta sizer). Size of snps was found to range between 100 to 998 nm. Results are shown below.

Results

			Diam. (nm)	% Intensity	Width (nm)
Z-Average (d.nm):	2366	Peak 1:	758.1	100.0	70.56
PdI:	0.799	Peak 2:	0.000	0.0	0.000
Intercept:	0.995	Peak 3:	0.000	0.0	0.000

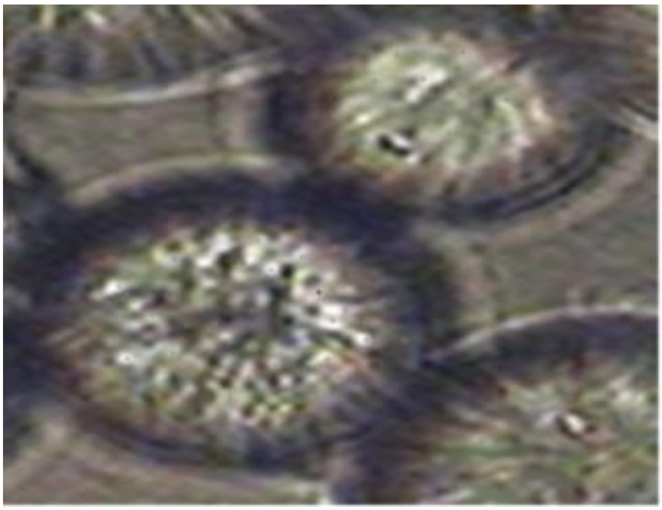
Result quality : Refer to quality report



For microsponges

A) **Microscopic analysis-**

To observe shape of microsponges phase contrast microscope was used, microsponges were found to be spherical in shape.



Evaluation of silver nanoparticles incorporated in microsponges

A) **Percent silver nanoparticle loaded in microsponges**

Percent of silver nanoparticles in prepared formulations was found to be 30 %.

B) **Percent yield**

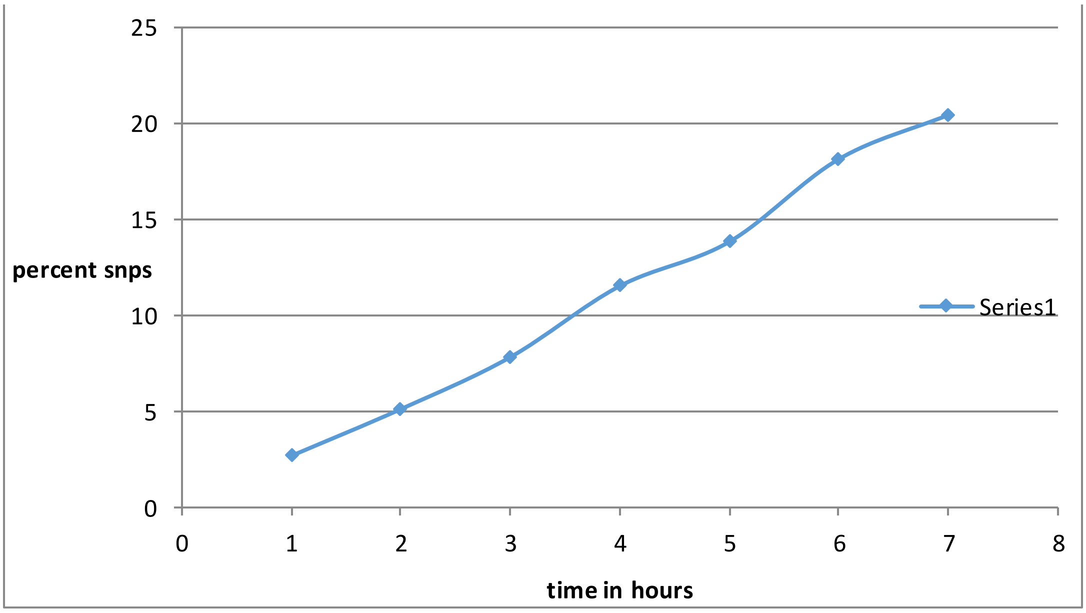
The percentage yield was calculated 70.37 %.

C) **In vitro release studies**

The release profile of silver nanoparticles-microsponges is shown in following figure and the data is given below in Table. The formulations exhibited sustained release profile over the period of time.

TIME(hours)	%CDR
1	2.67
2	5.1
3	7.8
4	11.5
5	13.8
6	18.1
7	20.4

Release study of silver nanoparticle-microsponge



DISCUSSION

Formulation of nanoparticles

Silver nanoparticles were prepared successfully using trisodium citrate and silver nitrate. Completion of reaction was characterized by color change, which was further confirmed by recording uv spectra.

Characterization of prepared nanoparticles

A) **Size distribution and zeta potential**

The prepared nanoparticles were characterized for size and shape. The size of nanoparticles was found to range between 100-998 nm as shown in Figure. Distribution in size of nanoparticles was found to be wide in range; this might have occurred due to aggregation of particles. Size distribution of silver nanoparticles was found to range between 100-998 nm.

B) **UV- Vis spectroscopy**

λmax of prepared nanoparticles was found to be 370 nm; which confirmed the formation of silver nanoparticles due to reduction of $AgNO_3$ with tri sodium citrate.

Characterization of microsponges

A) **Microscopic analysis-**

Shape of microsponges was analyzed with phase contrast microscope; microsponges were found to be spherical in shape, possessing pores which confirmed formation of sponge.

Evaluation of adsorbed nanoparticles on microsponges

A) **Percent loading**

The percentage of silver nanoparticles loaded onto microsponges was found to be reasonable. Amount of silver nanoparticles which was lost might have occurred due to saturation of pores with silver nanoparticles.

B) **Percent yield**

The yield of silver nanoparticles-microsponge formulation was found to be reasonable. The loss of yield might have occurred due to recovery problem and adherence of formulation due to sticky nature of polymer.

C) **In vitro release studies**

The release profile of silver nanoparticles from silver nanoparticles-microsponge formulation shows that the formulation exhibited sustained release profile.

CONCLUSION

Silver nanoparticles are good antimicrobial and anti-inflammatory agents. But may cause toxicity due to excess of silver ions in body; by entrapping silver nanoparticles in microsponges the release of silver nanoparticles can be controlled to reduce its toxicity. Controlled release microsponges of silver nanoparticles were successfully prepared and release of silver nanoparticles was controlled successfully. Toxic potential of these microsponges needs to be evaluated in future.

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