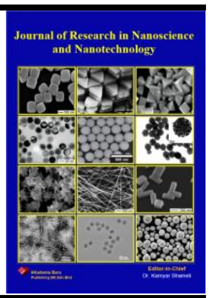
Penerbit Akademia Baru

Journal of Research in
Nanoscience and Nanotechnology

Journal homepage:
https://www.akademiabaru.com/submit/index.php/jrnn/
ISSN: 2773-6180




# Gamma Irradiation-Assisted Synthesis of Silver Nanoparticle and Their Antimicrobial Applications: A Review

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https://doi.org/10.37934/jrnn.3.1.5375

**ABSTRACT**

This review presents an introduction to the synthesis of silver nanoparticles (Ag-NPs) by gamma irradiation method. This method offers some benefits over the conventional methods because it provides fully reduced and highly pure nanoparticles free from by-products or chemical reducing agents, and is capable of controlling the particle size and structure. The nucleation and growth mechanism of metallic nanoparticles are also discussed. The competition between nucleation and growth process in the formation of nanoparticles can determine the size of nanoparticles which is influenced by certain parameters such as the choice of solvents and stabilizer, the precursor to stabilizer ratio, pH during synthesis, and absorbed dose. The present review, summarizes the gamma irradiation synthesis of Ag-NPs procedure, advantages, applications and their antibacterial properties.

*Keywords:*
Gamma irradiation; Silver nanoparticles; Antimicrobial, Chemical reduction method.

**Received: 28 May 2021** **Revised: 22 June 2021** **Accepted: 29 June 2021** **Published: 7 August 2021**

## 1. Introduction

Nanoparticles (NPs) are diversely ultra-structured particles either engineered and manufactured which have one dimension within the nano-scale diameters within the range from 1 to 100 nm [1, 2]. NPs stands for the billionth part of a meter, numerically written as $1\times10^{-9}$ meter with unique properties such as size-dependent qualities, high surface-to-volume ratio and unique optical properties at a critical length scale [3, 4]. As the size of NPs decrease, the surface area and energy increase; causing major changes to the physical, chemical and biological properties of from their conventional properties as bulk materials due to quantum effects [5–9]. Wider surface area provides sufficient binding sites for chemical reaction which confers the increase in catalytic activity [10, 11] as well as increases effective interaction with the microorganisms leading to high antimicrobial behavior.

The unique characteristics of NPs such as size, surface area, shapes, composition, determines their function and applications. such as sensors biomaterials [12], antimicrobial/antiseptic, medical [13–15]. Henceforth, the interest in preparing NPs designed to meet specific needs by surface modifications, controlling of their elementary size, shape and morphologies, have captured the interest of many to explore further [16].

Lately, incorporation of inorganic metal NPs into polymer matrix has received widespread attraction as the resulting metal nanocomposites possess superior strength and improved performance to better extent with affordable cost and wider applications. Metals NPs exhibit remarkable physical, chemical, and biological properties and are being explored as a promising approach to combating resistance to antibiotic. Since they effectively bridge bulk materials and atomic or molecular structures, metal NPs are now an important area of scientific research.

## 2. Silver Nanoparticles

Silver nanoparticles (Ag-NPs) are among the most favored metal NPs due to the multiple functionality as pronounced antimicrobial agent [17–19], combined with antifungal effect of Ag-NPs on dermatophytes [20, 21], anti-cancer [22], anti-inflammatory [23], antiviral [24], anti-angiogenesis [25] and antiplatelet activities [26], has made them the most used NPs to treat various diseases and infections [27] making them significant in the field of medicine and health care. Ag-NPs also demonstrated good catalytic properties in the field of dye detoxification, heavy metal removal and remediation [28, 29]. Ag-NPs has been explored widely and used in various industrial applications and incorporated into a variety of household and consumer products including textiles, cosmetics, paints, coatings, sensor, agricultural and food packaging, with a relatively high usage compared to other metals and alkaline earth metals in NPs applications [30] (Figure 1). The optical properties of metal NPs commonly used in various biochemical applications for food safety monitoring [127] and marine toxin detection [32]. They also have demonstrated excellent catalytic activities [33] and due to their exclusive structures and properties [34], and extensively applied in the field of homo and heterogeneous catalysis [35–37]. Due to the combination of small size with a large surface area, Ag-NPs have strong adsorption reactivity and capacity [38-40], thus Ag-NPs are promising tools for applicability in various wastewater ecosystems.

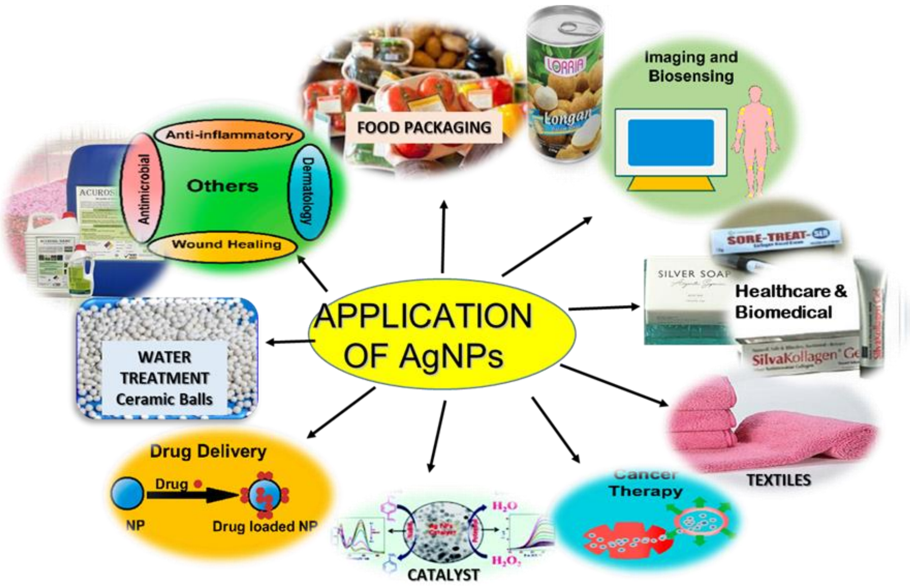


**Figure 1:** Various applications of Ag-NPs.

The biggest contribution (54%) of Ag-NPs are in the health and fitness, followed by cleaning and disinfecting (11%), food (10%), household equipment (6%), medicine (4%), electronic devices (3%) and others (12%) [41]. Their antimicrobial properties [42–44] are widely used in medicine sector such as antibacterial sprays, medicine, wound dressings [45], respirators, and heart valves [46–50]. Ag-NPs have also exhibited antimalarial [51], antiviral [52], anticancer [53–55], anti-plasmodial [51], larvicidal [56], antifungal [57, 54, 42, 58, 59], biofilm forming bacteria [60, 61], and pupicidal activity [62], disinfections, water and air purification, agriculture [63, 64], etc. These properties of silver encourage its use in, cosmetics, clothing and numerous household products, contraconceptives, detergent, dietary supplements, cutting boards, socks, shoes, cell phones, laptop keyboards, and children's toys [65, 66]. Ag-NPs are one of the most promising system to fight the emergence of pathogenic bacterial strains and the increase of resistant microorganisms resulted from the excessive use of antibiotics [67]. Nanotechnology products database 2019 reported, Ag-NCs have been used in 278 different types of product in 34 countries and 15 industries with diverse properties and applications [68, 69].

The specific properties of NPs such as size, shape, charge, and the composition material of the nanomaterial determine its specific activity [70]. Silver NPs can now be engineered to provide unique functionalities such as larger surface to volume ratio resulting into higher surface exposure and an increased rate of interaction between the test subjects and the ionic silver [71, 45, 50, 72–74].

## 3. Polymeric Support

The only limitation is small size Ag-NPs tends to agglomerate in solution (Figure 2), which will reduce their surface area hence deteriorate its capability to be used as an adsorbent, catalyst, antibacterial activity [75-77]. This can be resolved by adding a substrate which will promote strong interaction among the substrate and the NPs, reducing agglomeration effectively, thus enhancing their catalytic activity [78-79]. Therefor Ag metals are usually dispersed on a suitable support with large surface area for easy recovery and to enhance the dispersity and stability of Ag-NCs [80-84].

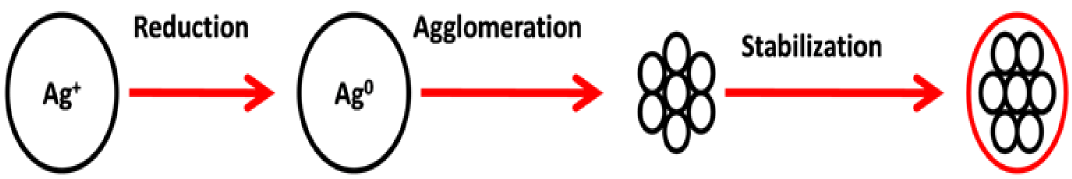


**Figure 2**: Synthesis mechanism of Ag-NPs.

The addition of supports is highly recommended to prevent agglomeration and as a stabilizer by providing a barrier and in controlling both the size and shape, and the final distribution of NPs [85–87]. The selection of the support material is critical as it may enhance or reduce the efficiency of Ag-NPs [88, 89]. Many researches focused on the stabilization of Ag on adsorbent materials, including cellulose fiber [90], polyethersulfone microfiltration membranes [91], clay [92], and activated carbon [93]. Clay materials are promising matrices for the preparation of Ag-NPs as they are available abundantly, cheap, good adsorbant, high specific surface area and ion exchange capacity, chemical stability and reusability [94–96]. Anchoring Ag-NPs on the surface of Kaolinite (Kln) is expected to be an economical and efficient catalyst for decomposition of organic contaminants [97]. One of the most effective and promising biomaterials are NCs based on Ag-NPs are Chitosan (Cts) [98] due to their significant antimicrobial activities against Gram-negative and Gram-positive bacteria and low toxicity toward mammalian cells [99-100].

## 4. Synthesis of Silver Nanocomposites

Synthesis of Ag-NPs such as the choice of the reducing and stabilizing agent play a major role in determining NPs with controlled structures and applications. The characteristic feature of nanomaterials, are continually developed in comparison to other NPs due to its significant antibacterial properties [101, 102]. Ag-NPs can be synthesized by different techniques, such as evaporation-condensation [103], laser ablation [104], gamma irradiation [105], lithography [106], micro-emulsion techniques [107], microwave-assisted technique [108] and biosynthesis using plants [109]. Generally, three different methods of synthesis used are physical, chemical, and biological method [110, 111]. However, the most common method used by the industry for production of Ag-NCs are the chemical methods.

## 5. Chemical Synthesis of Nanoparticles

Among the existing methods, synthesis by chemical reduction have been enormously employed and preferred for large quantities production of the NPs within short duration, specific size and shape [112–114]. The advantage of the chemical synthesis are pure Ag-NCs can be produced at low cost because of its low instrument requisites, minuscule duration and excessive yield. However, the disadvantage of the chemical method is high consumption of hazardous chemicals which are harmful to living organisms and waste disposal to the environment [115, 116]. Basically, the reduction of silver salts involves three main components, such as metal precursors, reducing agents, and stabilizing/capping agents. The most commonly used reducing agents are sodium borohydride, sodium hydroxide, sodium citrate, hydrazine hydrate, tollens reagents, N, N-dimethylformamide, poly(ethylene glycol)-block copolymers and ammonium formate [117–119].

In a chemical reaction, an oxidizing agent gains electrons and is itself reduced whereas a reducing agent loses electrons and is oxidized. Ag-NCs were prepared by mixing $AgNO_3$ a precursor with sodium borohydride ($NaBH_4$), a reducing agent. The silver ions ($Ag^+$) gain electrons and are reduced to silver atoms ($Ag^o$). The atoms will agglomerate into oligomeric clusters, which will eventually lead to the formation of Ag-NCs.

$$Ag^+ + e^-_{aq} \xrightarrow{\text{reduction}} Ag^0 \quad (1)$$

$$Ag^+ + H^0 \longrightarrow Ag^0 + H^+ \quad (2)$$

$$AgNO_3 + NaBH_4 \rightarrow Ag + H_2 + B_2H_6 + NaNO_3 \quad (3)$$

A stabilizer is usually added to prevent agglomeration and control the formation and stabilize the NCs. Additionally, the NCs produced by chemical synthesis will be sedimented with chemicals and are not applicable for biomedical application [120].

## 6. Gamma Irradiation Synthesis of Silver Nanoparticles

Gamma (γ) irradiation is a mature and powerful technology to be considered for the synthesis of Ag-NPs. Irradiation technique is considered a "green technique" as it does not require chemical initiator for reduction unlike the conventional methods [121]. It is a simple, clean process which can be performed using aqueous system, at ambient pressure and room temperature. The reduction of metal NPs by γ-irradiation is controlled by the dose of irradiation while the NPs produced are very pure, fully reduced without traced of chemical [122]. γ-radiation is a promising green technique for producing a wide range of materials, particularly polymeric materials and suitable for large-scale production [123]. Polyvinylpyrrolidone /carboxylmethyl cellulose prepared by γ-radiation increases the swelling and improves the water retention capability which governs the slow release of the hydrogels bringing release rate of urea 10 times higher than that of phosphate [124]. The reduction of Ag ions $Ag^{+}$) to Ag atoms ($Ag^{o}$) was achieved by the reducing species in the mixture solution.

When irradiation is applied to metal ions in an aqueous solution, irradiation energy is absorbed in water, which causes radiolysis of water and several products are generated, including hydrated electrons ($e_{aq}^{-}$), radical hydrogen atoms (H•) and radical hydroxyl (OH•) which are dispersed uniformly in the reaction medium (Eq. 1) [125]. The radiolytic method via γ-radiation is a powerful technique which can produce the desired morphology and distribution of metal NCs by adjusting the dose of irradiation [126]. In the aqueous solution, $AgNO_3$ separates to Ag+ and $NO_3^{-}$ ions as shown in Eq. 2. The powerful reductant generated from the radiolytic process, such as active electrons $e_{aq}^{-}$ and H atoms (H•), will reduce silver ions ($Ag^{+}$) to the zerovalent state ($Ag^{0}$) (Eqs. 3-4).

$$n{*}H_2O \xrightarrow{\text{irradiation}} e^{-}_{aq} + OH\bullet + H\bullet \quad (1)$$

$$AgNO_3 \longrightarrow Ag^{+} + NO_3^{-} \quad (2)$$

$$Ag^{+} + e_{aq}^{-} \xrightarrow{\text{reduction}} Ag^{0} \quad (3)$$

$$Ag^{+} + H\bullet \longrightarrow Ag^{0} + H^{+} \quad (4)$$

The reduced neutral silver atom Ag0 tend to interact with other metal ions to form relatively stable Ag clusters which combines with neighbouring silver atoms and progressively grew into large clusters to form spherical Ag-NCs [127, 128] as follows (Eqs. 5-8):

$$Ag^{0} + Ag^{+} \longrightarrow Ag^{2+\bullet} \quad (5)$$

$$Ag^{2+\bullet} + Ag^{+} \longrightarrow Ag_3^{2+\bullet} \quad (6)$$

$$Ag_2^{+\bullet} + Ag_2^{+\bullet} \longrightarrow Ag_4^{2+} \quad (7)$$

$$(Ag)_n^{+} + ne^{-}_{aq}\, n^{-} \longrightarrow (Ag)_n \quad (8)$$

Where:

$(Ag)_n$ = Silver nanocluster containing "n" silver atom

$e_{aq}^{-}$ = Aqueous electron   hydrogen atoms (H•)

These processes occur simultaneously and the morphology (particle size and shape) and stability of the resulting Ag-NPs depends highly on the $AgNO_3$ concentration, carrier or stabilizer, irradiation dose and the synthesis route selected for reduction.

## 7. Advantages of Synthesis by Gamma Irradiation

Gamma (γ) irradiation is simple, rapid and environmental friendly strategy to uniformly penetrate thick layers of materials or organic matters without noticeable decay of the organic matrix [121]. Irradiation was also demonstrated to be an effective method to increase the kinetics of metal ion reduction at ambient temperature without excessive reducing agent thus leading to a more environmentally friendly process to generate metal NPs [129]. $\gamma$-radiation techniques produce pure Ag-NPs with narrow particle size distribution, homogenous and instantaneous without needing clean up or purification as there are no pollutants, providing a clean alternative over chemical and physicochemical methods [130]. This methodology has therefore been shown to be a powerful approach revolutionary techniques that provides unique advantages over conventional techniques [131].

Specifically, gamma radiation is well known to induce gelatin crosslinking and afford a three-dimensional network by forming chemical bonds [5] between molecular backbones [6]. Advantages of radiation-induced polymer degradation include its ability to promote reproducible and quantitative changes without the introduction of chemical reagents and concomitantly occurring product sterilization [7]. In addition to crosslinking, the incorporation of natural fibers (such as cellulose) into gelatin helps to improve the mechanical and thermal properties of the corresponding hydrogels. Generally, radicals are formed from chain scission of the polymer chain in high-energy crosslinking, especially when irradiating dried gelatin or cellulose in in the presence of oxygen.

## 8. Gamma Irradiation-Assisted Synthesis of Cellulose Nanocrystal-Reinforced Gelatine Hydrogels

It was observed that for NCs smaller than 100 nm, sphere shape NCs displayed higher cellular uptake than nanorods while for NCs larger than 100 nm nanorods exhibits higher cellular uptake followed by spheres [26]. Spherical Ag-NCs, with size within 10 -15 nm possess antiplatelet properties [26] and are less toxic [132]. Frankova *et al*. reported, spherical Ag-NCs with an average size of 10 nm exhibit biocompatibility for fibroblasts and keratinocytes [133].

## 9. Effected of Ag Ions Concentration to the Controlling Size of Ag-NPs

The increase in particle size at higher precursor concentration is also observed in many cases. By increasing the concentration of metal ions, final size of Ag-NCs increases due to the rate of ion association that forms larger particles increases. The sizes of NCs are much smaller than those obtained by Hamouda *et al.* [134], which was in the range of 8-17 nm for Ag-NPs from chemical synthesis while 9-21 nm in size for biological synthesis. While Balakarthikeyan *et al*. confirmed the nano size between 68 to 100 nm obtained from synthesis using $NaBH_4$ [135]. Ag-NPs synthesized chemically using PVP as stabilizer reported NCs of 20.4 nm in size [136]. Green synthesis utilizing Citrullus lanatus fruit rind as a reductant and capping agent yields stable, spherical Ag-NPs with an average diameter of 17.96 ± 0.16 nm [110]. Ag-NCs reduced using $NaBH_4$ showed the formation of cubical NCs with size range from 22-28 nm and 56-72 nm for biological synthesis [60]. Moosa *et al*. also reported an increase in the size of silver kaolinite nanocomposites (Ag/Kln NCs) with the increase in silver nitrate concentration. Similar observation was reported for silver kaolinite NPs synthesized using gamma irradiation. As the

## 9. Antimicrobial Activity of Ag-NPs

Historically, silver was considered an effective antibacterial substance before the invention of antibiotics and used to prevent inflammation and infections when treating battle wounds during the First World War [45]. Silver compounds displayed strong antibacterial against a wide range of antibacterial spectrum [101], antiviral, antifungal, biocidal and anti-inflammatory activities [137, 138], and are being incorporated into composite for biomedical applications as a better therapeutic strategy. Previous studies have reported the powerful antimicrobial properties of Ag-NPs against various multidrug resistant strains like Pseudomonas aeruginosa, ampicillin-resistant E. coli, erythromycin-resistant Streptococcus pyogenes, methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *S. aureus* (VRSA) [139, 140] and other eukaryotic microorganisms as compared to other metals. Ag-NPs were being also found to be effective for the inhibition of several viruses including hepatitis B virus, respiratory syncytial virus, herpes simplex virus type, monkey pox virus, [141], HIV-1 virus [142, 143] and a large number of fungi Aspergillum, Candida and Saccharomyces. The intrinsic cytotoxic property of Ag-NCs has been applied against various types of cancer cells, such as hepatocellular carcinoma, lung and breast cancer and cervical carcinoma [144].

It is believed, Ag-NPs anchor to the surface of the bacterial cell wall and penetrate it to cause structural changes to the membrane or increase its permeability, all of which trigger cells to die. Once in the cell, silver ions interact with thiol groups in critical bacterial enzymes and proteins, disrupting the respiration and metabolic pathway. This subsequently damage DNA and its production cycle, leading to cell death [142-145]. The antimicrobial process is explained in Figure 3.

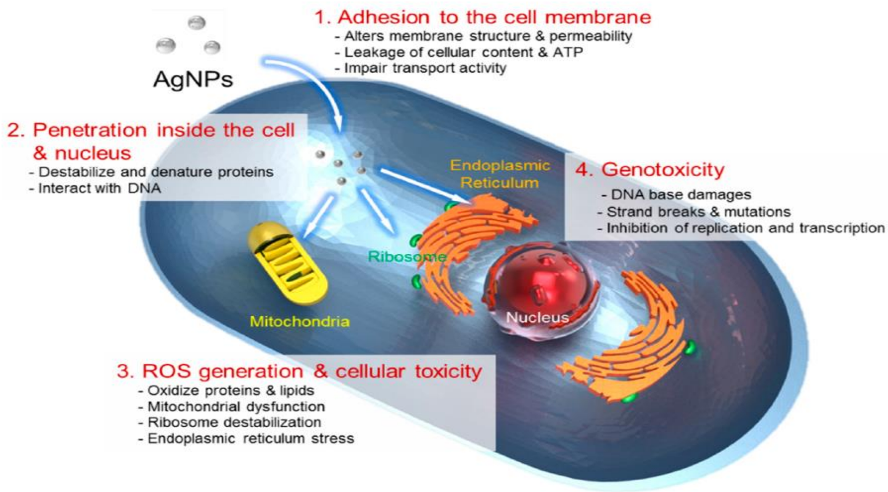


**Figure 3.** The four most prominent routes of antimicrobial action of Ag-NPs.

Some of the factors that influence the antimicrobial properties of Ag-NCs are shape, size, surface charge, capping agent, and synthesis method [146-147]. Size of NPs plays an important role to their antibacterial activity and typically, NPs with size below 50 nm, showed enhanced antimicrobial activity [147]. Smaller NPs, have relatively larger surface area and therefore an enlarged contact area which enhance their biological and chemical activities and increase the release rate of silver ion to enhance antimicrobial activities [148, 149]. Ag-NPs within 1 to 30 nm size are the most commonly used range and were found to be optimal against *S. aureus* and Klebsiella pneumonia, while 10–15 nm range sizes have highest antimicrobial activity. It was observed that Ag-NPs of size 22.5 nm enhance the antibacterial activity of penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin [65]. Studies on poly(acrylamide/itaconic acid) as a stabilizer for prepared of Ag-NPs by gamma radiation demonstrated that size were 50 to 42 nm when the irradiation dose is increased from 20 to 50 kGy [150]. While other studies show increase in size with the increase of irradiation dose.

Beside the size, electrostatic interaction caused by difference of charge between the negatively charged microorganism cell membrane and the positively charged Ag-NPs, plays an important role [151]. The difference of charge causes the Ag-NCs to be accumulated on the cell membrane, puncture the bacterial cell wall by reacting with the peptidoglycan component, and releasing silver ions into the bacterial cell [152]. The shape of NPs equally accelerates the rate of ion release, studies indicated that spherical and triangular shapes NP seem to have higher antimicrobial activity, while other studies showed truncated triangular Ag-NPs showed the most grounded antibacterial action. The incorporation of silver into a larger number of materials resulted in 95% reduction of antimicrobial activity. Fabrics are now incorporated with engineered Ag-NCs to kill odor-causing bacteria in socks and sports clothing, to prevent microbial spreading as in wound dressing, bedsheet, hospital uniform. including plastics, coatings, and foams as well as natural and synthetic fibers [153]. Food packaging with coatings of Ag-NPs displayed good inhibition against L. *mono cytogenes*, *E. coli, P. citrinum, S. aureus* and *A. niger*, among others resulting in significant reduction of mold and coliforms, increasing shelf life and keeping vegetable, bread and orange juice longer without reducing nutritional values, colour, consistency, flavour and taste the food fresh [154–156]. Ag-NCs deposited into guar gum and on film exhibited improved optical, spectral, thermo-mechanical, oxygen barrier and antimicrobial properties of film for active food packaging applications. In animal husbandry, Ag-NPs are used as a disinfectant to treat against poultry disease caused by yeast from infected cow udders and caused by biological material via eggs, chicken etc. Due to the exceptional antimicrobial properties of Ag-NPs toward a wide range of microorganisms, fungi and viruses, several Ag based medical products have been developed to control microbial proliferation, such as topical creams, antiseptic sprays, cancer therapeutics, pharmaceutical, dentistry, medical devices, bandages, wound healing dressings, disinfectant, bio-imaging and bio-sensing, being efficiently implemented [157]. $AgNO_3$ and silver sulfadiazine are used to prevent bacterial growth in drinking water, sterilizations and burn care [54]. Ag-NCs were found to produce free radicals which will leads to apoptosis resulting in necrobiosis. In low concentrations, Ag has been indicated as non-toxic material to humans, and it has been assessed as a promising material in pharmaceutical and biomedical fields [158], great potential for reducing infections and provides faster healing and better health to the patients [159]. The Ag-NPs with Cts bead hydrogel applied in drug delivery displayed enhanced antibacterial activity against *E. coli* and *S. aureus* with controlled and prolonged drug release observed [160].

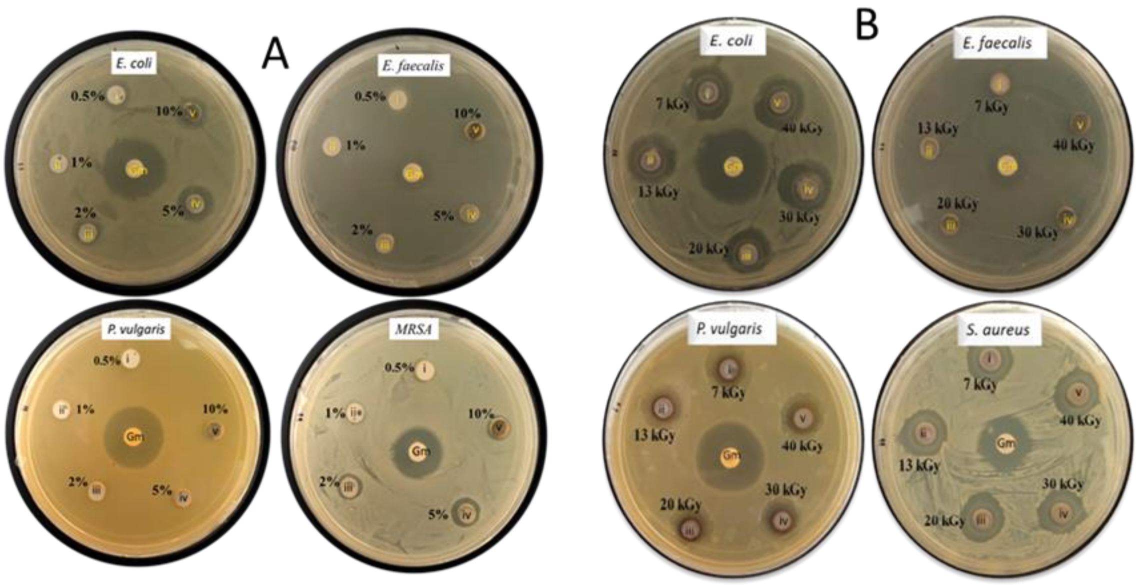


**Figure 4.** Inhibition zone of Ag/Kln synthesised using **A.** Chemical reduction at 0.5%, 1 %, 2 %, 5 % and 10 % **B.** Gamma reduction at 7, 13, 20, 30, and 40, towards *E. coli*, *E. faecalis*, *P. vulgaris* and MRSA. Gentamicin (Gm, 10 µg/ml) and Nystatin (10 µg/ml) is used as positive control.

In a study by S. Moosa *et al* [161], Ag/Kln NCs synthesized using chemical method and gamma irradiation were evaluated against *E. coli, S. aureus, E. faecalis and P. vulgaris* and *C. albicans*. The chemical synthesis Ag/Kln NPs, displayed minimum antimicrobial properties (Figure 4A) compared to the gamma synthesized Ag/Kln NPs (Figure 4B). The strong hydrogen bond network in the Kln matrix prevents the release of Ag+ in order to react with bacteria and also hinders any form of intercalation [162, 163]. Meanwhile, the gamma synthesized NPs displayed excellent antimicrobial properties compared to chemically synthesized NPs. Gamma ray irradiation induces the chain scission as well as breaking H bonds and releasing Ag+ ions [164]. This suggests that the synthesis method potentially affects the ability of Ag-NCs to release Ag+ ions which is also an important factor in determining antimicrobial activity, besides size, shape and structure. From this study, it was observed that the smallest NPs which correspond with Ag/Kln NPs synthesized chemically do not show the best inhibitory effects because of factors preventing Ag+ ions release, the main responsible for bacterial inhibition.

It was apparent that the gamma synthesized Ag/Kln NCs displayed significantly ($p < 0.05$) highest antibacterial activity than the chemically synthesized Ag/Kln NCs and the gamma synthesized Ag/Kln/Cts BNCs as well towards all the pathogens. The most susceptible bacterial strains were E. coli, followed by *P. vulgaris, MRSA, E. faecalis* and *C. albicans* with zones of inhibition of 17.3, 15.7, 14.7, 11.7 and 15.0 mm, respectively. The remarkably high antimicrobial activity of the gamma synthesized Ag/Kln NCs could be attributed to the release of sufficient silver ions from the Kln surface. $\gamma$-irradiation induces the chain scission as well as breaking the strong H bonds tightly interlinking the Kln lamellae and releasing Ag+ ions from the tightly bound clay surface [164]. This is evident to conclude that the prepared hybrid Ag/Kln NCs exhibits strong antibacterial activity which can be used as antimicrobial agent for biomedical and food industrial applications. Hence, Ag/Kln-NCs is a promising antimicrobial agent to work with the merits of both advantages of antibacterial and optical enhancing properties, high surface area, strong permeability and low toxicity to the human body and therefore, Ag-NCs constitute a very promising approach for the development of new antimicrobial systems. The literature compiled in Table 1 confirms the potential antimicrobial properties of Ag-NPs synthesis by gamma irradiation with various matrix as support, in a wide range of applications.

The polyvinyl alcohol/sodium alginate/Ag-NPs composite films upon exposure to various gamma irradiation doses of 5 to 15 kGy exhibited increased of antibacterial activities against S. aureus and *E. coli* [165]. Ag-NPs coating on cotton fabric prohibited Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and Candida albicans microbes from proliferating on fabric surface [128]. Study by Gharab *et al* where two types of preparation where the first preparation using silver, chitosan, citrus pectin, sodium alginate were irradiated at 5 kGy and second preparation using *Pleurotus ostreatus* was irradiated at 20 kGy. The synthesized Ag-NPs have potential effect as antioxidant and inhibit cancer cell. Potential Ag-NPs comprising silver chitosan polyethylene oxide synthesized using 20 kGy gamma irradiation have antibacterial activity against gram-negative (*Esherichia coli*) and gram-positive bacterium (Staphylococcus aureus) [166].

Ag-NPs synthesized with low molecular weight chitosan by gamma irradiation inhibited the growth of Methicillin-resistant *S. aureus* (MRSA) and *Aeromonas hydrophila* bacteria [167]. Silver nanoparticles prepared by $\gamma$-ray irradiation at 10–25 kGy using chitosan as a stabilizer formed Ag chitosan NPs exhibited inhibitory activity against *E. coli* and *S. aureus* [168]. $\gamma$-irradiation is useful for mass production of Ag-NPs using green techniques, especially in biomedicine. Colloidal Ag chitosan NPs exhibited highly antimicrobial effect against *S. aureus* and *Corticium salmonicolor* [169]. Polyvinyl alcohol/sodium alginate/nano silver (PVA/SA/Ag) composite films by in situ gamma irradiation displayed effective inhibition of Staphylococcus aureus, methicillin-resistant *S. aureus* [170]. Biosynthesis of Ag-NPs from *Enterococcus sp.* Culture displayed excellent enhanced antimicrobial activity than the commercial antibiotics and potential to inhibit the cell viability of liver cancer cells lines (HepG2) and lung cancer cell lines (A549). Ag-NPs/chitosan synthesized by γ-irradiation showed strong inhibition against *C. cassiicola* and reduce disease incidence of rubber leaves infected by *C. cassiicola*. [171]

**Characterization of Silver Nanoparticles**

The reduction of silver ions was monitored by visual observation of colour change and was further confirmed by sharp peaks shown by the absorption spectrum recorded by using UV-Vis spectrophotometer. It is well known that Ag-NCs exhibit yellowish-brown color in aqueous solution due to the excitation of surface plasmon resonance (SPR) band in the UV-visible region [173]. This was observed by Moosa *et al*., in the synthesis of Ag-NPs in kaolinite, upon the addition of $NaBH_4$, the color of the Ag/Kln suspension changed instantly to yellow then to yellowish-brown, subsequently to brown, indicating the formation of Ag-NPs in the kaolinite suspension where Ag+ was reduced to Ag0 (Figure 5). As the intensities of $AgNO_3$ increase, so does the intensity of the color of the suspension increases from yellow to brown and darker indicating the formation of Ag/Kln NCs in the kaolinite suspension. Fig 5, shows the colour change of Ag/Kln-NCs suspension from white to yellow, then to yellowish-brown, brown and darker brown upon addition of $NaBH_4$.


**Figure 5.** Photograph of Ag/Kln NCs suspension at different $AgNO_3$ concentrations synthesized chemically and using gamma irradiation.

Similar to the synthesis of Ag/Kln NCs, Aqueous suspensions containing Kln and silver nitrate ($1×10^{-2}$ M $AgNO_3$), when exposed to different γ-irradiation doses 0, 7, 13, 20, 30, 40, 65 and 80 kGy, displayed a spectrum of greyish to black colors, depending on the absorbed doses. Both chemically synthesized Ag/Kln NCs using $NaBH_4$ as a reductant agent yielded smaller Ag-NCs than those obtained using gamma irradiation. Beside the synthesis method, the concentration of silver and the irradiation dose also affect the size of NCs. It was revealed that as the concentration of silver or the irradiation dose is increased, the size of the NCs increased as well due to an increase in the reduction of $Ag^+$ and formation of NCs.

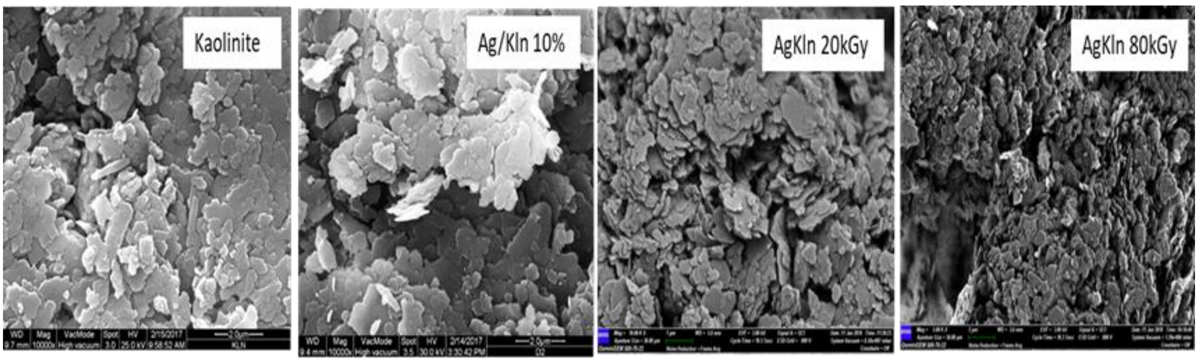


**Figure 6.** FESEM image of Kle, Ag/Kln 10% synthesized using $NaBH_4$, Ag/Kln 20 kGy and Ag/Kln 80kGy.

**Table 1.** Antimicrobial of Ag-NPs synthesize by gamma irradiation and their applications.

| Ag-NCs of different matrix | Size | Antimicrobial properties and application | References |
|---|---|---|---|
| Polyvinyl alcohol/sodium algi-nate/nano silver (PVA/SA/Ag) composite films by in situ gamma irradiation | ▪ 80 and 100nm | ▪ The composite films exhibited increased of antibacterial activities against *S. aureus* and *E. coli* upon exposure to various doses of gamma irradiation.<br>▪ The size of the NPs is reduced from 100 to 80 nm when the irradiation dose increases from 5 kGy to 15 kGy. | [165] |
| Poly(Acrylamide/Itaconic Acid)–Ag-NPs | ▪ Spherical<br>▪ 42 to 50 nm | ▪ Excellent antibacterial property against strains of Pseudomonas aeruginosa and slightly active against Escherichia coli, *methicillin-resistant Staphylococcus aureus*, and *Klebsiella pneumonia*. and 20, 30, 40, 50 and 70 kGy | [150] |
| Colloidal Ag-NPs in a water isopropanol polyvinylalcohol system | • Spherical<br>• 30 nm | ▪ Ag-NPs prepared using 60 Co-gamma radiation at total dose of 35 kGy on cotton fabric prohibited Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli and Candida albicans microbes from proliferating on fabric surface. | [128] |
| Ag-NPs synthesized using<br>i. Chitosan, citrus pectin and sodium alginate<br>ii Pleurotus ostreatus. | • 26 nm - 5 nm | • Synthesized Ag-NPs citrus pectin at 5.0 kGy and fermented fenugreek powder at 20.0 kGy have potential effect as antioxidant and inhibit cancer cell. | [122] |
| Ag chitosan polyethylene oxide nanocomposites prepared using gamma irradiation at 20 kGy | NA | ▪ Ag/Cts and Ag/Cts/PEO nanocomposites have antibacterial activity against gram-negative (Esherichia coli) and gram-positive bacterium (*Staphylococcus aureus*). | [166] |
| Synthesis of Ag-NPs with low molecular weight chitosan by gamma irradiation at 16 and 40 kGy | ▪ Spherical<br>▪ 5–30 nm | ▪ The resulting Ag/Cts NPs inhibited the growth of *Methicillin-resistant S. aureus* (MRSA) and *Aeromonas hydrophila* bacteria. | [167] |
| Ag chitosan NPs were prepared by 25 kGy γ-ray irradiation | spherical<br>7–30 nm | Ag chitosan NPs exhibited inhibitory activity against *E. coli* and *S. aureus.* The γ-ray doses of | [168] |
| Colloidal Ag-NPs using chitosan as a stabiliser and free radical scavenger | 7 nm | Colloidal Ag chitosan NPs exhibited highly antimicrobial effect against *S. aureus.* and *Corticium salmonicolor* | [169] |

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| Ag/poly(vinyl alcohol) hydrogels prepared using gamma irradiation at 25 kGy dose. | | Remarkable antibacterial activity Ag/PVA hydrogel against *Escherichia coli* and S. aureus *bacteria*. | [172] |
| Ag-NP/gelatin/PVA irradiated at 30, 40, or 50 kGy | ▪ 8 nm | Effective inhibition of *Staphylococcus aureus, methicillin-resistant S. aureus*. The degree of crosslink of the hydrogels increased with the increase in the irradiation dose and decrease with an increase in the amount of $AgNO_3$. | [170] |
| biosynthesis of eco-friendly Ag-NPs using culture supernatant of Enterococcus sp. | ▪ Spherical<br>▪ 10 – 80nm | ▪ Excellent enhanced antimicrobial activity than the commercial antibiotics<br>Ag-NPs have the great potential to inhibit the cell viability of liver cancer cells lines (HepG2) and lung cancer cell lines (A549). | [53] |
| Ag kaolinite matrices synthesized:<br>i. using chemical and γ- irradiation | ▪ Spherical<br>▪ 0.95 – 16 nm | Excelleny microbial properties against the gram-positive strains (*S. aureus and E. faecalis*) and gram-negative bacterial strains (*P. vulgaris and E. coli*), and yeast *C. Albicans* | [161] |
| Synthesis of Ag-NPs/Chitosan by $\gamma$- Irradiation from 8 to 28 kGy | ▪ 15 to 5 nm | ▪ Ag-NPs inhibited the growth of *Corynespora cassiicola* on rubber-leaf extract media | [171] |

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FESEM was employed to determine the surface morphology of the developed Ag/Kln NCs. The energies of the emitted x-rays identify the element providing qualitative and quantitative information regarding the elemental ingredient of the NCs. The intensity of silver signal was intensified with the increased $AgNO_3$ concentration, and the dose of gamma irradiation, suggesting the formation of enhanced growth of Ag-NPs. No obvious peak belong to impurity is detected. The result indicates that the as synthesized Ag-NPs synthesized using gamma irradiation is composed of high purity Ag-NPs (Figure 6).

## Conclusion

Chemical synthesis is commonly favoured due to their simplicity and production of enormous quantity of monodispersed NCs possessing controlled size, shapes and morphologies However, synthesis using gamma irradiation technology is simple, safe and can be manufactured at a larger scale at room condition and under ambient pressure. Gamma synthesis is considered a green technology and environmentally benign without using harsh chemical as reducing agents making it a suitable candidate for medical, drug delivery antimicrobial and fungicidal agents in the future and dental applications. Beside the synthesis method, the concentration of silver and the irradiation dose also affect the size of NPs. It was revealed that as the concentration of silver or the irradiation dose is increased, the size of the NCs increased as well due to an increase in the reduction of $Ag^+$ and formation of NPs. The antimicrobial evaluation indicate that the Ag-NPs prepared by gamma irradiation displayed excellent antimicrobial activity compared to those obtained by chemical synthesis. This is evident to conclude that the Ag-NPs prepared by gamma irradiation exhibits pure and strong antibacterial activity which can be used as antimicrobial agent for biomedical and food industrial applications.

## Acknowledgement

This research was funded by Takasago Thermal Engineering Co. Ltd. grant (R.K.130000.7343.4B422) from the research management center (RMC) of Universiti Teknologi Malaysia (UTM) and Malaysia Japan International Institute of Technology (MJIIT).

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