

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

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### 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.

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### 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  $1 \times 10^{-9}$  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.

1 The unique characteristics of NPs such as size, surface area, shapes, composition, determines their  
2 function and applications. such as sensors biomaterials [12], antimicrobial/antiseptic, medical [13–  
3 15]. Henceforth, the interest in preparing NPs designed to meet specific needs by surface  
4 modifications, controlling of their elementary size, shape and morphologies, have captured the  
5 interest of many to explore further [16].

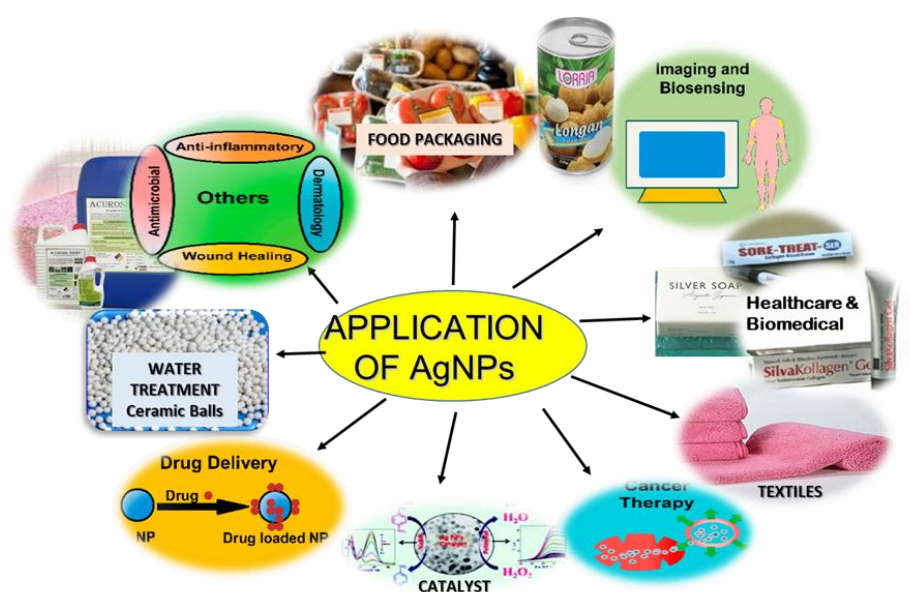
6 Lately, incorporation of inorganic metal NPs into polymer matrix has received widespread  
7 attraction as the resulting metal nanocomposites possess superior strength and improved  
8 performance to better extent with affordable cost and wider applications. Metals NPs exhibit  
9 remarkable physical,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

12

## 13 2. Silver Nanoparticles

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

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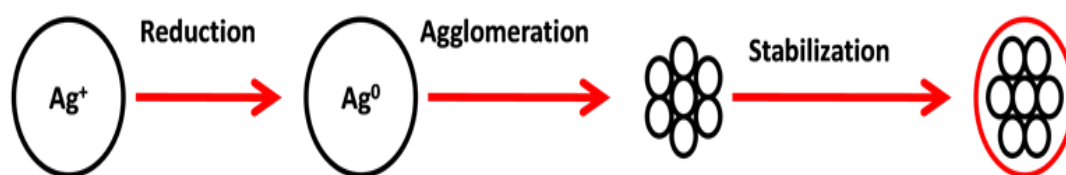
Figure 1: Various applications of Ag-NPs.

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

14 The specific properties of NPs such as size, shape, charge, and the composition material of the  
15 nanomaterial determine its specific activity [70]. Silver NPs can now be engineered to provide unique  
16 functionalities such as larger surface to volume ratio resulting into higher surface exposure and an  
17 increased rate of interaction between the test subjects and the ionic silver [71, 45, 50, 72–74].  
18

### 19 3. Polymeric Support

20 The only limitation is small size Ag-NPs tends to agglomerate in solution (Figure 2), which will  
21 reduce their surface area hence deteriorate its capability to be used as an adsorbent, catalyst,  
22 antibacterial activity [75-77]. This can be resolved by adding a substrate which will promote strong  
23 interaction among the substrate and the NPs, reducing agglomeration effectively, thus enhancing  
24 their catalytic activity [78-79]. Therefore Ag metals are usually dispersed on a suitable support with  
25 large surface area for easy recovery and to enhance the dispersity and stability of Ag-NCs [80-84].  
26



27  
28 **Figure 2:** Synthesis mechanism of Ag-NPs.

29 The addition of supports is highly recommended to prevent agglomeration and as a stabilizer by  
30 providing a barrier and in controlling both the size and shape, and the final distribution of NPs [85–  
31 87]. The selection of the support material is critical as it may enhance or reduce the efficiency of Ag-  
32 NPs [88, 89]. Many researches focused on the stabilization of Ag on adsorbent materials, including  
33 cellulose fiber [90], polyethersulfone microfiltration membranes [91], clay [92], and activated carbon  
34 [93]. Clay materials are promising matrices for the preparation of Ag-NPs as they are available  
35 abundantly, cheap, good adsorbent, high specific surface area and ion exchange capacity, chemical  
36 stability and reusability [94–96]. Anchoring Ag-NPs on the surface of Kaolinite (Kln) is expected to  
37 be an economical and efficient catalyst for decomposition of organic contaminants [97]. One of the  
38 most effective and promising biomaterials are NCs based on Ag-NPs are Chitosan (Cts) [98] due to

1 their significant antimicrobial activities against Gram-negative and Gram-positive bacteria and low  
2 toxicity toward mammalian cells [99-100].

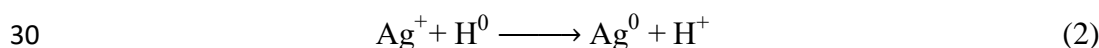
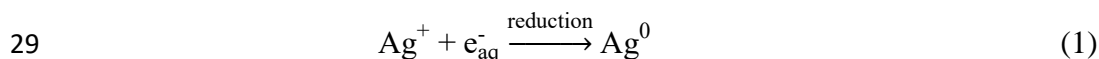
#### 3 **4. Synthesis of Silver Nanocomposites**

4 Synthesis of Ag-NPs such as the choice of the reducing and stabilizing agent play a major role in  
5 determining NPs with controlled structures and applications. The characteristic feature of  
6 nanomaterials, are continually developed in comparison to other NPs due to its significant  
7 antibacterial properties [101, 102]. Ag-NPs can be synthesized by different techniques, such as  
8 evaporation-condensation [103], laser ablation [104], gamma irradiation [105], lithography [106],  
9 micro-emulsion techniques [107], microwave-assisted technique [108] and biosynthesis using plants  
10 [109]. Generally, three different methods of synthesis used are physical, chemical, and biological  
11 method [110, 111]. However, the most common method used by the industry for production of Ag-  
12 NCs are the chemical methods.

#### 13 **5. Chemical Synthesis of Nanoparticles**

14 Among the existing methods, synthesis by chemical reduction have been enormously employed  
15 and preferred for large quantities production of the NPs within short duration, specific size and shape  
16 [112–114]. The advantage of the chemical synthesis are pure Ag-NCs can be produced at low cost  
17 because of its low instrument requisites, minuscule duration and excessive yield. However, the  
18 disadvantage of the chemical method is high consumption of hazardous chemicals which are harmful  
19 to living organisms and waste disposal to the environment [115, 116]. Basically, the reduction of silver  
20 salts involves three main components, such as metal precursors, reducing agents, and  
21 stabilizing/capping agents. The most commonly used reducing agents are sodium borohydride,  
22 sodium hydroxide, sodium citrate, hydrazine hydrate, tollens reagents, N, N-dimethylformamide,  
23 poly(ethylene glycol)-block copolymers and ammonium formate [117–119].

24 In a chemical reaction, an oxidizing agent gains electrons and is itself reduced whereas a reducing  
25 agent loses electrons and is oxidized. Ag-NCs were prepared by mixing AgNO<sub>3</sub> a precursor with  
26 sodium borohydride (NaBH<sub>4</sub>), a reducing agent. The silver ions (Ag<sup>+</sup>) gain electrons and are reduced  
27 to silver atoms (Ag<sup>0</sup>). The atoms will agglomerate into oligomeric clusters, which will eventually lead  
28 to the formation of Ag-NCs.



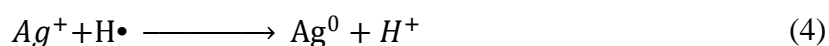
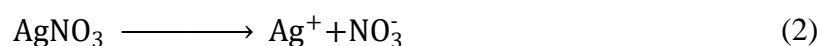
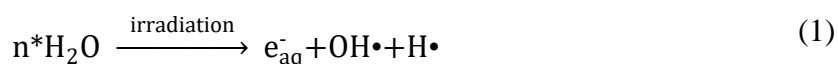
32 A stabilizer is usually added to prevent agglomeration and control the formation and stabilize  
33 the NCs. Additionally, the NCs produced by chemical synthesis will be sedimented with chemicals  
34 and are not applicable for biomedical application [120].

#### 35 **6. Gamma Irradiation Synthesis of Silver Nanoparticles**

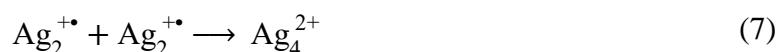
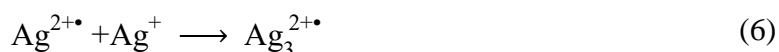
36 Gamma ( $\gamma$ ) irradiation is a mature and powerful technology to be considered for the synthesis of  
37 Ag-NPs. Irradiation technique is considered a “green technique” as it does not require chemical  
38 initiator for reduction unlike the conventional methods [121]. It is a simple, clean process which can  
39 be performed using aqueous system, at ambient pressure and room temperature. The reduction of

1 metal NPs by  $\gamma$ -irradiation is controlled by the dose of irradiation while the NPs produced are very  
2 pure, fully reduced without traced of chemical [122].  $\gamma$ -radiation is a promising green technique for  
3 producing a wide range of materials, particularly polymeric materials and suitable for large-scale  
4 production [123]. Polyvinylpyrrolidone /carboxymethyl cellulose prepared by  $\gamma$ -radiation increases  
5 the swelling and improves the water retention capability which governs the slow release of the  
6 hydrogels bringing release rate of urea 10 times higher than that of phosphate [124]. The reduction of  
7 Ag ions ( $Ag^+$ ) to Ag atoms ( $Ag^0$ ) was achieved by the reducing species in the mixture solution.

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



16 The reduced neutral silver atom  $Ag^0$  tend to interact with other metal ions to form relatively  
17 stable Ag clusters which combines with neighbouring silver atoms and progressively grew into large  
18 clusters to form spherical Ag-NCs [127, 128] as follows (Eqs. 5-8):



19 Where:

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

21  $e_{aq}^-$  = Aqueous electron hydrogen atoms ( $H\bullet$ )

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

## 25 7. Advantages of Synthesis by Gamma Irradiation

26 Gamma ( $\gamma$ ) irradiation is simple, rapid and environmental friendly strategy to uniformly  
27 penetrate thick layers of materials or organic matters without noticeable decay of the organic matrix  
28 [121]. Irradiation was also demonstrated to be an effective method to increase the kinetics of metal  
29 ion reduction at ambient temperature without excessive reducing agent thus leading to a more



1 environmentally friendly process to generate metal NPs [129].  $\gamma$ -radiation techniques produce pure  
2 Ag-NPs with narrow particle size distribution, homogenous and instantaneous without needing  
3 clean up or purification as there are no pollutants, providing a clean alternative over chemical and  
4 physicochemical methods [130]. This methodology has therefore been shown to be a powerful  
5 approach revolutionary techniques that provides unique advantages over conventional techniques  
6 [131].

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

## 15 **8. Gamma Irradiation-Assisted Synthesis of Cellulose Nanocrystal-Reinforced Gelatine** 16 **Hydrogels**

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

## 22 **9. Effect of Ag Ions Concentration to the Controlling Size of Ag-NPs**

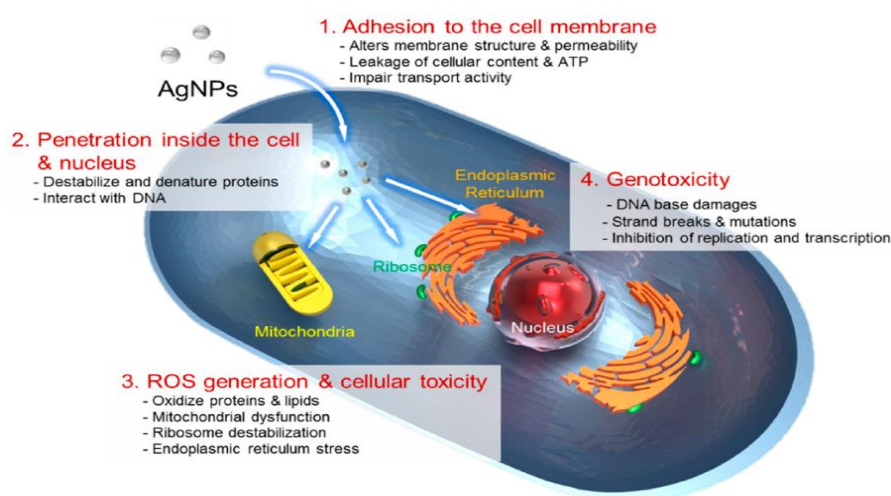
23 The increase in particle size at higher precursor concentration is also observed in many cases. By  
24 increasing the concentration of metal ions, final size of Ag-NCs increases due to the rate of ion  
25 association that forms larger particles increases. The sizes of NCs are much smaller than those  
26 obtained by Hamouda *et al.* [134], which was in the range of 8-17 nm for Ag-NPs from chemical  
27 synthesis while 9-21 nm in size for biological synthesis. While Balakarthikeyan *et al.* confirmed the  
28 nano size between 68 to 100 nm obtained from synthesis using  $\text{NaBH}_4$  [135]. Ag-NPs synthesized  
29 chemically using PVP as stabilizer reported NCs of 20.4 nm in size [136]. Green synthesis utilizing  
30 Citrullus lanatus fruit rind as a reductant and capping agent yields stable, spherical Ag-NPs with an  
31 average diameter of  $17.96 \pm 0.16$  nm [110]. Ag-NCs reduced using  $\text{NaBH}_4$  showed the formation of  
32 cubical NCs with size range from 22-28 nm and 56-72 nm for biological synthesis [60]. Moosa *et al.*  
33 also reported an increase in the size of silver kaolinite nanocomposites (Ag/KIn NCs) with the increase  
34 in silver nitrate concentration. Similar observation was reported for silver kaolinite NPs synthesized  
35 using gamma irradiation. As the

## 36 **9. Antimicrobial Activity of Ag-NPs**

37 Historically, silver was considered an effective antibacterial substance before the invention of  
38 antibiotics and used to prevent inflammation and infections when treating battle wounds during the  
39 First World War [45]. Silver compounds displayed strong antibacterial against a wide range of  
40 antibacterial spectrum [101], antiviral, antifungal, biocidal and anti-inflammatory activities [137, 138],  
41 and are being incorporated into composite for biomedical applications as a better therapeutic  
42 strategy. Previous studies have reported the powerful antimicrobial properties of Ag-NPs against

1 various multidrug resistant strains like *Pseudomonas aeruginosa*, ampicillin-resistant *E. coli*,  
2 erythromycin-resistant *Streptococcus pyogenes*, methicillin-resistant *S. aureus* (MRSA), vancomycin-  
3 resistant *S. aureus* (VRSA) [139, 140] and other eukaryotic microorganisms as compared to other  
4 metals. Ag-NPs were being also found to be effective for the inhibition of several viruses including  
5 hepatitis B virus, respiratory syncytial virus, herpes simplex virus type, monkey pox virus, [141],  
6 HIV-1 virus [142, 143] and a large number of fungi *Aspergillum*, *Candida* and *Saccharomyces*. The  
7 intrinsic cytotoxic property of Ag-NPs has been applied against various types of cancer cells, such as  
8 hepatocellular carcinoma, lung and breast cancer and cervical carcinoma [144].

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



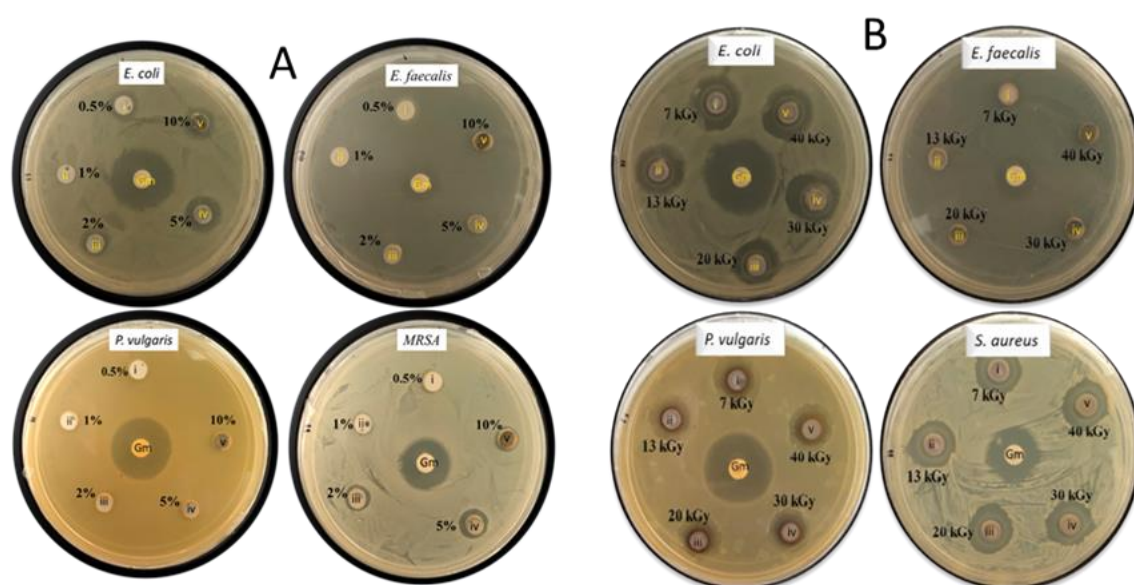
14

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

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

29 Beside the size, electrostatic interaction caused by difference of charge between the negatively  
30 charged microorganism cell membrane and the positively charged Ag-NPs, plays an important role  
31 [151]. The difference of charge causes the Ag-NPs to be accumulated on the cell membrane, puncture  
32 the bacterial cell wall by reacting with the peptidoglycan component, and releasing silver ions into  
33 the bacterial cell [152]. The shape of NPs equally accelerates the rate of ion release, studies indicated

1 that spherical and triangular shapes NP seem to have higher antimicrobial activity, while other  
2 studies showed truncated triangular Ag-NPs showed the most grounded antibacterial action. The  
3 incorporation of silver into a larger number of materials resulted in 95% reduction of antimicrobial  
4 activity. Fabrics are now incorporated with engineered Ag-NCs to kill odor-causing bacteria in socks  
5 and sports clothing, to prevent microbial spreading as in wound dressing, bedsheets, hospital uniforms,  
6 including plastics, coatings, and foams as well as natural and synthetic fibers [153]. Food packaging  
7 with coatings of Ag-NPs displayed good inhibition against *L. monocytogenes*, *E. coli*, *P. citrinum*, *S.*  
8 *aureus* and *A. niger*, among others resulting in significant reduction of mold and coliforms, increasing  
9 shelf life and keeping vegetable, bread and orange juice longer without reducing nutritional values,  
10 colour, consistency, flavour and taste the food fresh [154–156]. Ag-NCs deposited into guar gum and  
11 on film exhibited improved optical, spectral, thermo-mechanical, oxygen barrier and antimicrobial  
12 properties of film for active food packaging applications. In animal husbandry, Ag-NPs are used as  
13 a disinfectant to treat against poultry disease caused by yeast from infected cow udders and caused  
14 by biological material via eggs, chicken etc. Due to the exceptional antimicrobial properties of Ag-  
15 NPs toward a wide range of microorganisms, fungi and viruses, several Ag based medical products  
16 have been developed to control microbial proliferation, such as topical creams, antiseptic sprays,  
17 cancer therapeutics, pharmaceutical, dentistry, medical devices, bandages, wound healing dressings,  
18 disinfectant, bio-imaging and bio-sensing, being efficiently implemented [157]. AgNO<sub>3</sub> and silver  
19 sulfadiazine are used to prevent bacterial growth in drinking water, sterilizations and burn care [54].  
20 Ag-NCs were found to produce free radicals which will lead to apoptosis resulting in necrobiosis.  
21 In low concentrations, Ag has been indicated as non-toxic material to humans, and it has been  
22 assessed as a promising material in pharmaceutical and biomedical fields [158], great potential for  
23 reducing infections and provides faster healing and better health to the patients [159]. The Ag-NPs  
24 with Cts bead hydrogel applied in drug delivery displayed enhanced antibacterial activity against *E.*  
25 *coli* and *S. aureus* with controlled and prolonged drug release observed [160].



26

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

30 In a study by S. Moosa *et al* [161], Ag/KIn NCs synthesized using chemical method and gamma  
31 irradiation were evaluated against *E. coli*, *S. aureus*, *E. faecalis* and *P. vulgaris* and *C. albicans*. The



1 chemical synthesis Ag/KIn NPs, displayed minimum antimicrobial properties (Figure 4A) compared  
2 to the gamma synthesized Ag/KIn NPs (Figure 4B). The strong hydrogen bond network in the KIn  
3 matrix prevents the release of Ag<sup>+</sup> in order to react with bacteria and also hinders any form of  
4 intercalation [162, 163]. Meanwhile, the gamma synthesized NPs displayed excellent antimicrobial  
5 properties compared to chemically synthesized NPs. Gamma ray irradiation induces the chain  
6 scission as well as breaking H bonds and releasing Ag<sup>+</sup> ions [164]. This suggests that the synthesis  
7 method potentially affects the ability of Ag-NCs to release Ag<sup>+</sup> ions which is also an important factor  
8 in determining antimicrobial activity, besides size, shape and structure. From this study, it was  
9 observed that the smallest NPs which correspond with Ag/KIn NPs synthesized chemically do not  
10 show the best inhibitory effects because of factors preventing Ag<sup>+</sup> ions release, the main responsible  
11 for bacterial inhibition.

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

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

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

1 lines (HepG2) and lung cancer cell lines (A549). Ag-NPs/chitosan synthesized by  $\gamma$ -irradiation  
2 showed strong inhibition against *C. cassiicola* and reduce disease incidence of rubber leaves infected  
3 by *C. cassiicola*. [171]

#### 4 **Characterization of Silver Nanoparticles**

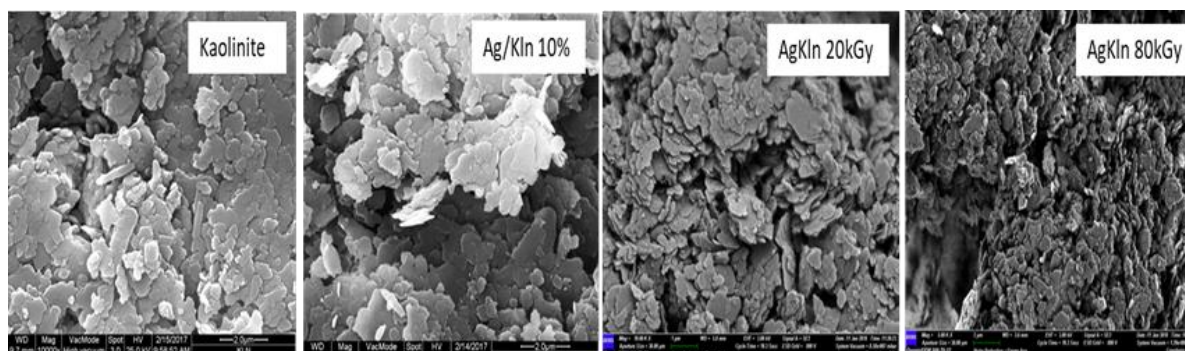
5 The reduction of silver ions was monitored by visual observation of colour change and was  
6 further confirmed by sharp peaks shown by the absorption spectrum recorded by using UV-Vis  
7 spectrophotometer. It is well known that Ag-NCs exhibit yellowish-brown color in aqueous solution  
8 due to the excitation of surface plasmon resonance (SPR) band in the UV-visible region [173]. This  
9 was observed by Moosa *et al.*, in the synthesis of Ag-NPs in kaolinite, upon the addition of  $\text{NaBH}_4$ ,  
10 the color of the Ag/Kln suspension changed instantly to yellow then to yellowish-brown,  
11 subsequently to brown, indicating the formation of Ag-NPs in the kaolinite suspension where  $\text{Ag}^+$   
12 was reduced to  $\text{Ag}^0$  (Figure 5). As the intensities of  $\text{AgNO}_3$  increase, so does the intensity of the color  
13 of the suspension increases from yellow to brown and darker indicating the formation of Ag/Kln NCs  
14 in the kaolinite suspension. Fig 5, shows the colour change of Ag/Kln-NCs suspension from white to  
15 yellow, then to yellowish-brown, brown and darker brown upon addition of  $\text{NaBH}_4$ .



16

17 **Figure 5.** Photograph of Ag/Kln NCs suspension at different  $\text{AgNO}_3$  concentrations synthesized  
18 chemically and using gamma irradiation.

19 Similar to the synthesis of Ag/Kln NCs, Aqueous suspensions containing Kln and silver nitrate  
20 ( $1 \times 10^{-2}$  M  $\text{AgNO}_3$ ), when exposed to different  $\gamma$ -irradiation doses 0, 7, 13, 20, 30, 40, 65 and 80 kGy,  
21 displayed a spectrum of greyish to black colors, depending on the absorbed doses. Both chemically  
22 synthesized Ag/Kln NCs using  $\text{NaBH}_4$  as a reductant agent yielded smaller Ag-NCs than those  
23 obtained using gamma irradiation. Beside the synthesis method, the concentration of silver and the  
24 irradiation dose also affect the size of NCs. It was revealed that as the concentration of silver or the  
25 irradiation dose is increased, the size of the NCs increased as well due to an increase in the reduction  
26 of  $\text{Ag}^+$  and formation of NCs.



27

28 **Figure 6.** FESEM image of Kln, Ag/Kln 10% synthesized using  $\text{NaBH}_4$ , Ag/Kln 20 kGy and Ag/Kln  
29 80kGy.

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

Ag-NCs of different matrix	Size	Antimicrobial properties and application	References
Polyvinyl alcohol/sodium alginate/nano silver (PVA/SA/Ag) composite films by in situ gamma irradiation	<ul style="list-style-type: none"> <li>▪ 80 and 100nm</li> </ul>	<ul style="list-style-type: none"> <li>▪ The composite films exhibited increased of antibacterial activities against <i>S. aureus</i> and <i>E. coli</i> upon exposure to various doses of gamma irradiation.</li> <li>▪ The size of the NPs is reduced from 100 to 80 nm when the irradiation dose increases from 5 kGy to 15 kGy.</li> </ul>	[165]
Poly(Acrylamide/Itaconic Acid)-Ag-NPs	<ul style="list-style-type: none"> <li>▪ Spherical</li> <li>▪ 42 to 50 nm</li> </ul>	<ul style="list-style-type: none"> <li>▪ Excellent antibacterial property against strains of <i>Pseudomonas aeruginosa</i> and slightly active against <i>Escherichia coli</i>, <i>methicillin-resistant Staphylococcus aureus</i>, and <i>Klebsiella pneumoniae</i>. and 20, 30, 40, 50 and 70 kGy</li> </ul>	[150]
Colloidal Ag-NPs in a water isopropanol polyvinylalcohol system	<ul style="list-style-type: none"> <li>• Spherical</li> <li>• 30 nm</li> </ul>	<ul style="list-style-type: none"> <li>▪ Ag-NPs prepared using 60 Co-gamma radiation at total dose of 35 kGy on cotton fabric prohibited <i>Pseudomonas aeruginosa</i>, <i>Staphylococcus aureus</i>, <i>Escherichia coli</i> and <i>Candida albicans</i> microbes from proliferating on fabric surface.</li> </ul>	[128]
Ag-NPs synthesized using i. Chitosan, citrus pectin and sodium alginate ii Pleurotus ostreatus.	<ul style="list-style-type: none"> <li>• 26 nm - 5 nm</li> </ul>	<ul style="list-style-type: none"> <li>• 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.</li> </ul>	[122]
Ag chitosan polyethylene oxide nanocomposites prepared using gamma irradiation at 20 kGy	NA	<ul style="list-style-type: none"> <li>▪ Ag/Cts and Ag/Cts/PEO nanocomposites have antibacterial activity against gram-negative (<i>Escherichia coli</i>) and gram-positive bacterium (<i>Staphylococcus aureus</i>).</li> </ul>	[166]
Synthesis of Ag-NPs with low molecular weight chitosan by gamma irradiation at 16 and 40 kGy	<ul style="list-style-type: none"> <li>▪ Spherical</li> <li>▪ 5–30 nm</li> </ul>	<ul style="list-style-type: none"> <li>▪ The resulting Ag/Cts NPs inhibited the growth of <i>Methicillin-resistant S. aureus</i> (MRSA) and <i>Aeromonas hydrophila</i> bacteria.</li> </ul>	[167]
Ag chitosan NPs were prepared by 25 kGy $\gamma$ -ray irradiation	spherical 7–30 nm	Ag chitosan NPs exhibited inhibitory activity against <i>E. coli</i> and <i>S. aureus</i> . The $\gamma$ -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 <i>S. aureus</i> . and <i>Corticium salmonicolor</i>	[169]

Ag/poly(vinyl alcohol) hydrogels prepared using gamma irradiation at 25 kGy dose.		Remarkable antibacterial activity Ag/PVA hydrogel against <i>Escherichia coli</i> and <i>S. aureus</i> bacteria.	[172]
Ag-NP/gelatin/PVA irradiated at 30, 40, or 50 kGy	<ul style="list-style-type: none"> <li>▪ 8 nm</li> </ul>	Effective inhibition of <i>Staphylococcus aureus</i> , methicillin-resistant <i>S. aureus</i> . 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 <sub>3</sub> .	[170]
biosynthesis of eco-friendly Ag-NPs using culture supernatant of <i>Enterococcus</i> sp.	<ul style="list-style-type: none"> <li>▪ Spherical</li> <li>▪ 10 – 80nm</li> </ul>	<ul style="list-style-type: none"> <li>▪ Excellent enhanced antimicrobial activity than the commercial antibiotics</li> <li>▪ Ag-NPs have the great potential to inhibit the cell viability of liver cancer cells lines (HepG2) and lung cancer cell lines (A549).</li> </ul>	[53]
Ag kaolinite matrices synthesized: i. using chemical and $\gamma$ - irradiation	<ul style="list-style-type: none"> <li>▪ Spherical</li> <li>▪ 0.95 – 16 nm</li> </ul>	Excelleny microbial properties against the gram-positive strains ( <i>S. aureus</i> and <i>E. faecalis</i> ) and gram-negative bacterial strains ( <i>P. vulgaris</i> and <i>E. coli</i> ), and yeast <i>C. Albicans</i>	[161]
Synthesis of Ag-NPs/Chitosan by $\gamma$ - Irradiation from 8 to 28 kGy	<ul style="list-style-type: none"> <li>▪ 15 to 5 nm</li> </ul>	<ul style="list-style-type: none"> <li>▪ Ag-NPs inhibited the growth of <i>Corynespora cassiicola</i> on rubber-leaf extract media</li> </ul>	[171]



FESEM was employed to determine the surface morphology of the developed Ag/KIn 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<sub>3</sub> 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<sup>+</sup> 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.

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