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Investigating the Application of Silver Nanoparticles in Active Food Packaging: Antimicrobial Properties and Synthesis Methods

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Abstract Nanotechnology has been used in various sciences since its introduction in 1959, and its new applications are being found in different industries every day. The food industry is one of the industries in which nanotechnology has an advancing trend. Nanotechnology has been applied in different sectors of this industry, such as food safety, food design, and food protection. Nowadays, scientists' attention has been paid to silver nanoparticles for being used in food packaging and food protection with an emphasis on their antimicrobial properties. The application of nanotechnology in food packaging is divided into four categories: reinforced packaging, active packaging, intelligent packaging, and biodegradable nano-composites packaging; the use of silver nanoparticles in packaging is in the field of active packaging. There are many hypotheses about how silver nanoparticles apply antimicrobial properties, the most probable of which is the binding of silver nanoparticles to thiol groups in the enzymes of organisms, which leads to the inactivation of enzymes and finally damage to the cell wall. In general, silver nanoparticles can extend the shelf life of food by prolonging the lag phase and suppressing the growth of pathogens. In general, three chemicals, physical and biological methods are employed to produce nanoparticles. Recently, the use of biological methods or green chemistry has received much attention for the production of nanoparticles since there is no need for high energy consumption and advanced equipment, and also the lack of damage to the environment.

Keywords Active packaging, Antimicrobial, Food packaging, Silver nanoparticles, Synthesis

Introduction

Nanotechnology is the science of manufacturing and developing materials and structures on the scale of a billionth of a meter. If a large molecule can be reduced to the scale of a nanometer, it leads to the development of particular chemical and physical properties specified for that nanoparticle that is considerably different from the larger properties of the original molecule. Despite the significant development of nanotechnology in many fields, the use of nanotechnology in food packaging is still in its infancy. Since an extensive range of nanomaterials with their functional properties can be used to enhance packaging, the future of food packaging can be attributed to this technology [1-5]. The first concept of nanotechnology was first introduced in 1959 by Nobel Laureate Richard Feynman, and the term nanotechnology was coined by Norio Taniguchi many years later in 1974. From the beginning, the role of nanotechnology in the packaging industry was considered to improve packaging capabilities.

The use of this technology in food packaging increases the quality of food, including its color, taste, texture stability, smell, and shelf life [6,7]. The reduction of food waste, which is one of the positive aspects of using nanotechnology in food packaging, is one of the main reasons for this increase. The Food and Agriculture Organization of the United Nations (FAO) estimated that one-third of the world's produced food is wasted in different ways. In the United States, the value of annually wasted food is equal to $165 billion, which is 40% of the food market's share and has adverse effects on the economy. This rate is equal to 50% of the produced food in the European Union [8-11]. The food packaging by controlling the transfer of moisture and gases as well as preventing the access of spoilage agents such as bacteria, fungi, and mold, play an important role in the safety and quality of food and the reduction of food waste [2,12]. Nanotechnology is expected to affect 25% of the food packaging market, which is currently estimated to be $100 billion in the next decade [13].

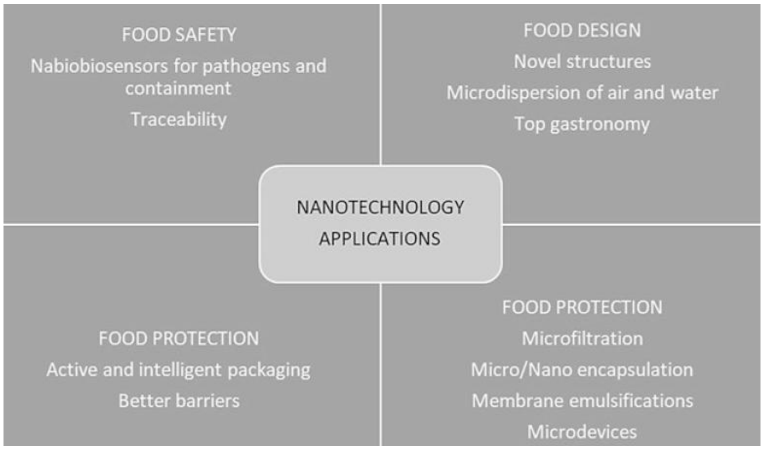

Figure 1: Some common applications of nanotechnology in the food industry [14]

Packaging means manufacturing or providing a container or protector that maintains the health of the product within the period from harvesting, production, transportation, storage, and distribution until final consumption and preserves it from possible physical or chemical hazards. Also, the packaging must be light and cost-effective. The purpose of food packaging is to preserve the product, prevent bacterial spoilage, increase shelf life, prevent damage during transportation, and storage. Food packaging makes a major contribution to safety and maintaining food quality and can control the transfer of moisture and gases; as a result, food waste is significantly reduced [15]. Mostly used materials in food packaging are metal, glass, and paper. Over the past decade, the use of polymers and plastics has replaced other types of food packaging because of cost-effectiveness, ductility, and variety in physical properties and has resulted in many developments in the food industry. Nowadays, plastic polymers are the most commonly used materials in food packaging, which are of non-biodegradable materials and cause irrecoverable damage to the environment. These undecomposed plastics pose a serious threat to humans and the environment. The time required to decompose various types of plastic is different. This period can be in the range of 15 years up to never. On the other hand, another major problem with these packages is the probable permeability of gas and other small molecules [16,17].

Materials and Methods

The present article is the result of investigating articles, books and conference papers related to the topic and keywords of research in various databases such as Google Scholar, Science Direct and other related databases.

Results & Discussion

Nano-packaging and the Role of Silver Nanoparticles

According to conducted investigations, there are four fields in the application of nanotechnology in food packaging: reinforced packaging, active packaging, intelligent packaging, and biodegradable nano-composites packaging [13]. The active packaging and intelligent packaging are often considered as one case. In the definition of intelligent packaging, it can be said that this packaging contains an internal or external identifier that informs the consumer about the storage conditions of the package and the quality of the food inside the package [18]. Intelligent packaging can also be considered as an advanced type of active packaging that provides the consumer with information about the quality of the product during the period of storage and distribution to the consumer while controlling the packaging conditions [19,20]. However, active packaging is a type of packaging that detects adverse alterations in the internal environment of the system and modifies until reaching the optimal conditions [19,20]. In contrast with traditional packaging that focuses on protection systems, active packaging refers to a system that leads to the direct release of antimicrobial substances on the food surface. It is also possible that antimicrobial agents to be released in the form of steam in the upper space of the package. Despite the higher efficiency of direct release of antimicrobial substances, in cases that direct release of antimicrobial substances on food surface is required, laboratory studies and further studies are needed before commercialization and market entry. Accordingly, edible films prevent the activity of pathogenic bacteria and spoilage of the product by developing an effective aggregation of antimicrobial substances on the surface of the food [21,22]. A large part of nanotechnology that used to reduce the wastes of food packaging is based on nanocomposite. Nanocomposites made from a combination of nanomaterials in plastic polymers have high flexibility and are resistant against temperature, moisture, and gas permeability. The use of nanocomposites can increase the application of edible and biodegradable films and the coating [23-25]. The high antimicrobial properties and low toxicity of free silver ions for mammalian cells have increased the application of this ion in active and intelligent nano-packaging [26]. The antimicrobial material used in active packaging should not have an adverse effect on the sensory properties of food. On the other hand, the release rate of antimicrobial material should be controlled since the over-release of antimicrobial material damages the food [27,28]. Silver can kill gram-positive and gram-negative bacteria and even viruses such as HIV with a much higher effect compared to disinfectants. The researches have indicated that silver can kill 650 types of germs without leaving any side effects, which is contrary to disinfectants that leave many side effects. The mentioned capability has led to the expansion of the use of silver in related industries [29,30].

Investigating the Antimicrobial Properties of Silver Nanoparticles

Since ancient civilizations, silver was used to cover the dishes to prevent contamination by germs. Silver was later found to be the most effective antibacterial agent with minimum toxicity for living cells [31]. In World War I, silver was used to prevent the growth of microbes in soldiers' wounds. Silver is currently used in various medical fields as a suitable barrier to prevent the growth of microbes [32]. Although the antimicrobial properties of silver have been used for centuries, its mechanism is unclear.

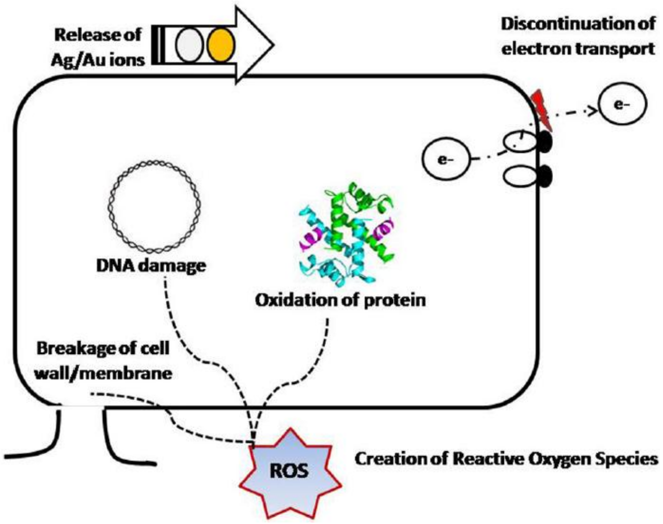

Figure 2: Different mechanisms of nanoparticles in the development of antimicrobial properties [6]

Deactivating the enzyme function in microorganisms is considered to be the most probable hypothesis for the antimicrobial function of silver ions. In this process, silver atoms deactivate the enzyme by binding to thiol groups in the enzyme. Thiols (-SH) are organic compounds similar to alcohols in which sulfur reacts with hydrocarbons instead of oxygen [33]. Throughout this process, silver replaces hydrogen in thiol groups, and an S-silver bond is formed in the cell membrane of enzymes. The formation of this bond eventually leads to the elimination of microorganisms [34].

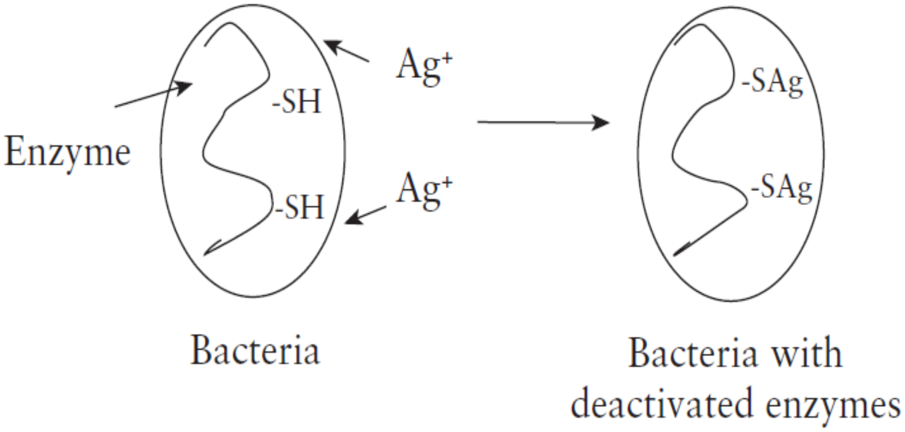

Figure 3: The reaction of silver nanoparticles with thiol groups [35]

The compounds containing thiol groups such as the amino acids with (SH) in their structure, such as cysteine, neutralize the antibacterial activity of silver. The amino acids with disulfide bonds and sulfur-containing compounds cannot neutralize the activity of silver ion [36]. The antimicrobial activity of silver appears to be on the basis of developing the structural and functional defects in bacterial cells. Silver ions have the capability of altering the permeability of a cell wall through binding to the cell wall, leading to the elimination of that. The binding of silver ions to cell membranes, DNA, bacterial proteins, and ribosomes are of other methods in which silver ions can apply their antimicrobial effect [37].

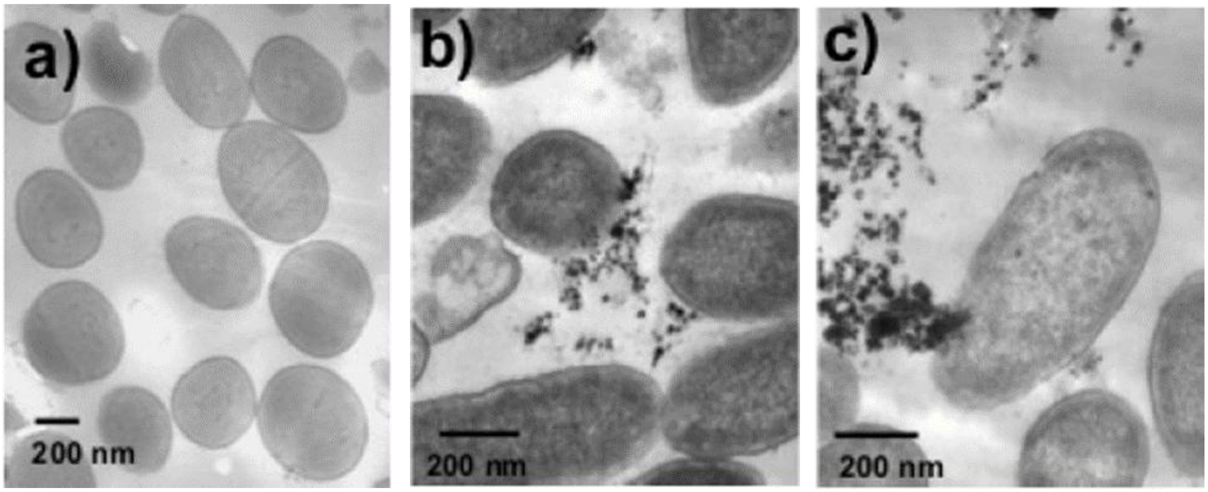

Figure 4: TEM images of a Pseudomonas aeruginosa sample at different magnifications are shown. (a) The control sample, i.e., no silver nanoparticles were used; (b) and (c) samples that were previously treated with silver nanoparticles [38]

The effect of silver nanocomposites is positive on an extensive range of pathogenic microorganisms, such as Escherichia coli [39-42], Staphylococcus aureus [43,44], Vibrio cholerae [45], and Pseudomonas aeruginosa [46]. In general, particles smaller than 10 nm are toxic for bacteria, including Escherichia coli and Pseudomonas aeruginosa [38]. High chemical reactivity and bioavailability increase the possibility of being toxicity in nanomaterials so that a similar volume of a substance in the nanoscale has a higher potential of toxicity compared to the larger scale of particles [14,47]. Silver nanoparticles have higher contact and significantly affect the microorganisms when they are separated. Over time, the interconnection of these particles is possible, which leads to the reduction of their effectiveness. According to previous research, silver nanoparticles with the size of 1-10 nanometers have the highest antimicrobial effect if they are separated and possess an excellent contact surface. It should be pointed out that silver particles can kill useful bacteria in food (fermented and probiotic foods) and in the human body; however, they usually do not have any effect on human cells at concentrations between 21 to 26 micrograms per liter [48-51]. Following EU rules, the allowed amount of silver ions in food is equal to 0.05 mg Ag/Kg [51]. The overall objective of active antimicrobial packaging is to prolong the lag phase and suppress the growth of microorganisms to increase the shelf life of food [52].

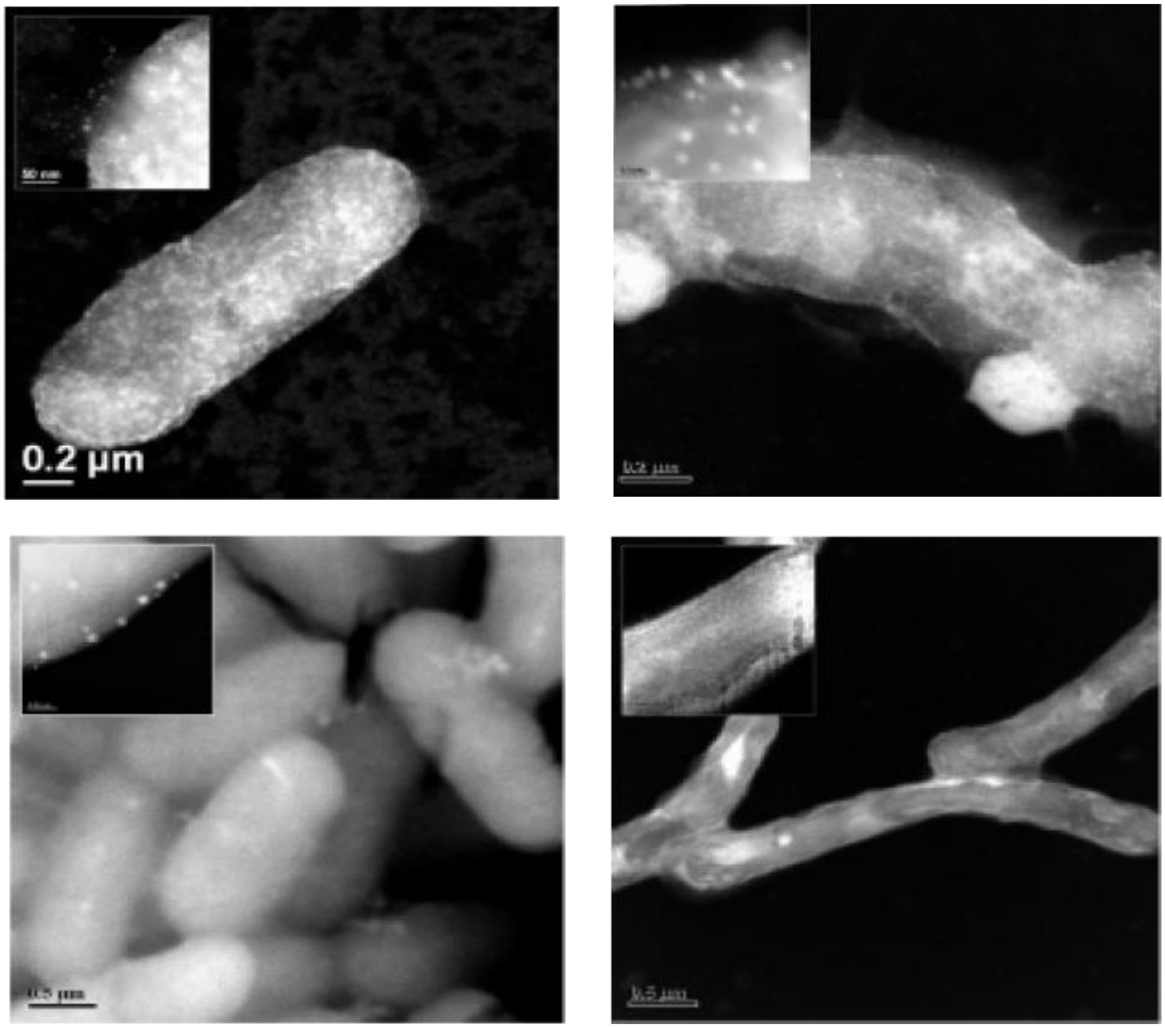

Figure 5: HAADF STEM images that show the interaction of the bacteria with the silver nanoparticles: (a) Escherichia coli, (b) Salmonella typhi, (c) Pseudomonas aeruginosa, and (d) Vibrio cholerae. [38]

In general, antimicrobial packaging can be divided into two general categories; in the first type, the antimicrobial agent exerts its effect by transmitting onto the surface of the food, while in the second type, the antimicrobial agent is not transmitted toward the food [53,54]. The antimicrobial function can be used in different ways in active food packaging. The direct mixture of volatile and non-volatile antimicrobial agents in polymers, adsorbing or coating of antimicrobial materials on polymer surfaces, immobilization of antimicrobial compounds in polymers by ionic or covalent bonds, the addition of sachets containing antimicrobial compounds in packaging, and the use of antimicrobial films are among these methods [55].

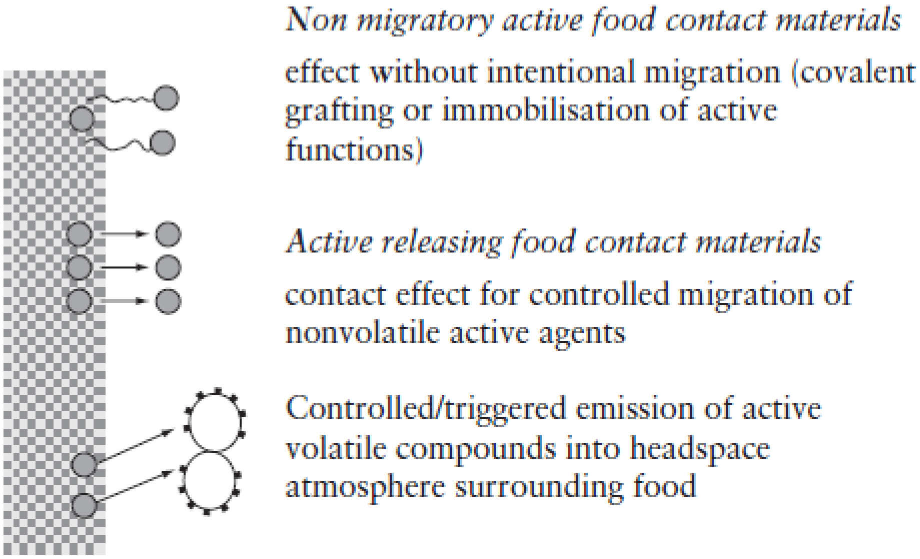

Figure 6: Different types of active food contact materials [35]

Other natural and synthetic antimicrobial compounds have previously been employed to protect food products from pathogens. The antimicrobial compounds such as sorbates, benzoates, propionates, and parabens can be used as food preservatives. In previous studies, sodium benzoate and potassium sorbate have been employed in chitosan film [56,57]. The bacteriocins are synthesized ribosomal bioactive peptide compounds in the form of a peptide complex or released on the extracellular surface that have a bacteriostatic effect on other species. The use of bacteriocins as biological preservatives has been started since about more than two decades ago. The molecular weight of these protein metabolites is usually less than 10 kDa. Nicin, lacticins, pediocin, diolococcin, enterocins, and propionicins are of these bacteriocins. It has been indicated in many studies that while Nisin affects Gram-positive organisms, it does not affect Gram-negative bacteria. This issue can be solved by adding food-grade Chelating agents such as EDTA (Ethylenediaminetetraacetic acid) and Citric acid [58,59]. Chitosan ($C6H11NO4$) is a substance with antimicrobial properties that is suitable for manufacturing film or coating and can act as a carrier for other additives [60-63]. Many studies have reported an increase in antimicrobial activity of chemical and natural antimicrobial agents as a result of mixing with silver nanoparticles. These mixed compounds can be suitable options for use in active packaging [64-67].

Different Methods of Synthesizing Silver Nanoparticles

The methods for producing silver nanoparticles are divided into three general categories: chemical, physical, and biological [68]. In the physical approach, physical energy is used to produce nanoparticles. Although this approach is the most appropriate method to produce silver nanoparticles in the form of powder and can be used to produce large volumes of nanoparticles in a single process, the cost of initial investment to purchase equipment must be considered [69]. In general, evaporation condensation is used to produce metal nanoparticles in the physical approach. Furthermore, silver nanoparticles are also obtained by laser ablation of metallic bulk materials in solution [70,71]. The chemical reduction method for producing nanoparticles is the most common method due to its ease and need for simple equipment. The size of the resulting nanoparticles is one of the most important factors that affect the selection of the proper method for synthesizing the nanoparticle. Companies and individuals tend to produce metal nanoparticles with controlled size.

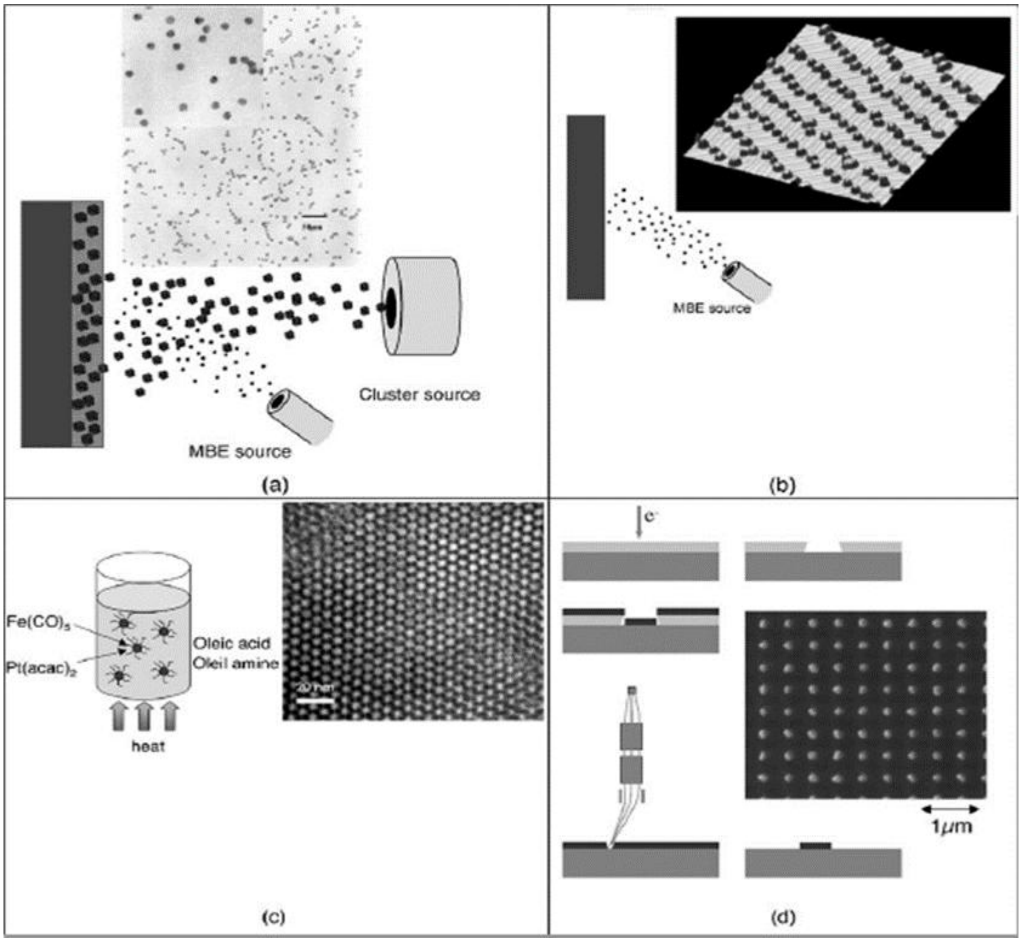

Figure 7: Generic methods for producing nanoparticles [72]

The general methods for producing nanoparticles are shown in Fig.7, in which the section indicates the production of pre-formed nanoparticles in the gas phase. All methods of producing gas-phase nanoparticles include the production of super-saturated metal vapor. These vapors condense into particles. This method, which is a physical approach, is referred to as the most flexible synthesis method. In this method, a tube furnace is often employed to evaporate at atmospheric pressure so that the source of the material is placed in the center of the furnace, and evaporation is done [69,72]. The excessive occupancy of space, high energy consumption, and a long time to achieve stable heat are the disadvantages of using a tube furnace [69]. As shown in section b of Fig.7, in another approach, nanoparticles are obtained by deposition of atomic vapor on surfaces. Optimizing the coverage, substrate temperature, and deposition rate are some of the ways to control the size of particles in this method [72]. Section c of Fig.7 indicates the wet chemical method. There are several methods to reduce metal salts and obtain nanoparticles as a suspension in the solvent. By placing a drop of this suspension on a surface under controlled evaporation conditions, the suspension can be condensed as a regular flat monolayer on a surface [72]. These three methods are known as bottom-up approaches, i.e., the nanoparticles are synthesized from atoms. Section d of Fig.7 shows top-down approaches. The top-down method separates nanoparticles from larger structures by employing the two main methods of electron beam lithography (EBL) and focused ion beam milling (FIB milling). Both of these methods have some limitations compared to bottom-up approaches so that they are unable to produce particles as small as the size of particles produced by bottom-up approaches. However, EBL and FIB milling are more flexible in terms of the morphology of nanoparticle and can produce particles of various morphologies [72].

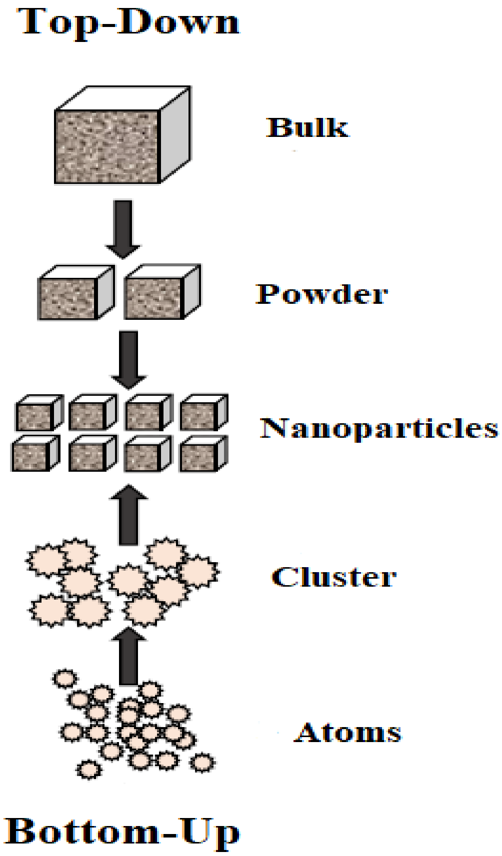

Figure 8: Top-down and Bottom-up approaches [73]

Recently, the use of naturally reducing agents such as polysaccharides and biological microorganisms (bacteria, fungus, and plant extracts) or green chemistry has received more attention in biosynthetic methods [69,74]. The development of biological processes for the synthesis of nanoparticles is evolving and has become an important branch of nanotechnology [75]. The biological production systems have received special attention because of their effectiveness and flexibility. The microbial cells are highly organized units that are capable of synthesizing renewable particles of specified size and structure due to their morphology and metabolic pathways. The biosynthetic nanoparticles often have water-soluble and biocompatible properties, which are appropriate for many applications and even necessary for some of them [76]. The high pressure, energy, temperature, and toxic chemicals are among other advantages of nanoparticle biosynthesis [77]. Table 1 lists some species of microorganisms that synthesize silver nanoparticles.

Table 1: Some species of microorganisms producing silver nanoparticles in different sizes

S. No	Organism	Size (nm)	Reference

	Bacteria		
1	*Pseudomonas stutzeri* AG259	200	[78]
2	*Lactobacillus Strains*	500	[79]
3	*Bacillus megaterium*	46.9	[80]
4	*Klebsiella pneumonia* (culture supernatant)	50	[81]
5	*Bacillus licheniformis*	50	[82]
6	*Bacillus licheniformis* (culture supernatant)	50	[83]
7	*Corynebacterium* sp.	10–15	[84]
8	*Bacillus subtilis* (culture supernatant)	5–60	[85]
9	*Geobacter sulfurreducens*	200	[**86**]
10	*Morganella* sp.	20 ± 5	[8**7**]
11	*Bacillus subtilis*	5–60	[8**5**]
12	*Escherichia coli*	1–100	**[88،89]**
13	*Proteus mirabilis*	10–20	[**90**]
14	*Bacillus* sp.	5–15	[**91**]
15	*Bacillus cereus*	4 & 5	[9**2**]
16	*Staphylococcus aureus*	1–100	[9**3**]
17	Lactic acid bacteria	11.2	[9**4**]
18	*Brevibacterium casei*	50	[9**5**]
19	*Plectonema boryanum*	1-200	[9**6**]
20	*Enterobacter cloacae*	50-100	[9**7**]
	Fungi		
1	*Fusarium oxysporum*	5–50	[**98**]
2	*Aspergillus fumigatus*	5–25	[**99**]
3	*Aspergillus niger*	20	[**100**]
4	*Phanerochaete chrysosporium*	100	[10**1**]
5	*Aspergillus flavus*	8.92 ± 1.61	[10**2**]
6	*Cladosporium cladosporioides*	10–100	[10**3**]
7	*Fusarium semitectum*	10–60	[10**4**]
8	*Trichoderma asperellum*	13–18	**[105،106]**
9	*Cladosporium cladosporioides*	10–100	[10**3**]
10	*Trichoderma viride*	5–40	[10**7**]
11	*Penicillium fellutanum*	1–100	[**108**]
12	*Penicillium brevicompactum* WA 2315	23–105	[**109**]
13	*Verticillium* sp.	25 ± 12	[**110**]
14	*Fusarium solani*	5–35	[11**1**]
15	*Fusarium acuminatum*	5–40	[11**2**]
16	*Aspergillus clavatus*	10–25	[11**3**]
17	*Phoma sp. 3.2883*	70	[11**4**]
18	*Coriolus versicolor*	350-600	[11**5**]
	Plants		
1	*Azadirachta indica*	50	[11**6**]
2	*Cinnamomum camphora* leaf	55–80	[11**7**]
3	*Glycine max* (soybean) leaf extract	25–100	[11**8**]
4	*Jatropha curcas*	10–20	[1**19**]
5	*Cinnamomum camphora* Leaf	5–40	[12**0**]
6	*Phyllanthus amarus*	18–38	[12**1**]
7	*Carica papaya*	60–80	[12**2**]
8	*Gliricidia sepium*	10–50	[12**3**]
9	*Coriandrum sativum* leaf extract	26	[12**4**]
10	*Camellia sinensis*	200	[12**5**]
11	*Medicago sativa*	2-20	[12**6**]
12	*Aloe vera*	15-20	[12**7**]
13	*Cinnamomum zeylanicum* bark	50-100	[12**8**]
14	*Desmodium triflorum*	5-20	[1**29**]
15	*Piper betle* leaf	3-37	[13**0**]

Conclusion

The use of this technology in food packaging is expanding every day with the introduction of various properties such as antimicrobial properties, heat and light resistance, and enhancing gas and heat resistance for packaging by nanoparticles. Therefore, further experiments are required to investigate the effect of these particles on human cells and the migration of nanoparticles to food. The migration of nanoparticles from packages to beverages or food is commonly cited as the major concern. Many investigations reveal that the smaller the nanoparticles and the lower the density, the higher the probability of these particles being transferred to food and causing health problems for the consumer.

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