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INVESTIGATION OF MECHANISM AND EFFECTIVENESS OF METAL NANOPARTICLES IN SELF-STERILIZING PACKAGING

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Abstract: *Microbial contaminants intimidate food safety and shelf-life. Metal nanoparticles (NPs) have become a leading area of interest and research in barrier packaging materials that ensure food safety. Traits such as small size, high surface-to-volume ratio and multi-functionality make them ideal materials for producing self-sterilizing packaging. Numerous metal NPs have proven to fight against a wide range of pathogenic microbes through various methods. Further, metal NPs exhibit more biocompatibility than metal ions. This study investigates the role and the mechanism of action of the various NPs in self-sterilizing packaging. AgNPs, TiO_2NPs, MgONPs, ZnONPs, AuNPs, FeONPs, Cu-based NPs and SnO_2NPs have been explored for their biocidal action in self-sterilizing surfaces and food packaging applications in this work. The size, shape, surface structure, surface reactivity and other environmental factors (like pH) influence the biocidal properties of these metal NPs. From the literature survey, it was inferred that it was necessary for the metal NPs to be smaller than 50 nm in size to exhibit effective biocidal action against pathogenic microbes. The mechanisms followed by the metal NPs against bacteria and fungi include disturbing the cell wall, the metabolic process by inducing reactive oxygen species (ROS) and/or the DNA synthesis mechanism. It was inferred that AgNPs, MgNPs and ZnONPs are some of the NPs that have a significant share in self-sterilizing surfaces. Being expensive, the works of literature on AuNPs and their application in this subject are very few. This paper aims to study the biocidal behaviour and rank the effectiveness of these metal NPs to act as ideal materials for self-sterilising packaging.*

Keywords: Metal nano particles, self-sterilizing packaging, nano technology, nano particle Synthesis

1. INTRODUCTION

In food products, microbial spoilage is one of the major reasons for food deterioration. The major factors affecting the growth of microorganisms are moisture content and pH of food, storage temperature, humidity and type of packaging. The microorganisms can be classified based on the temperature range in which they can survive and thrive. Psychrophiles or psychrotrophic bacteria can survive at very low temperatures from 0°C or lower to about 20°C whereas mesophilic bacteria propagate well between 20°C and 45°C. The microorganisms that grow above 45°C are called thermophiles. Moulds can adapt to a broader temperature range than bacteria while yeasts cannot survive in the thermophilic range. Yeasts generally grow in the psychrophilic and mesophilic temperature range. Food additives such as salt, sugar, acids and weak carboxylic acids such as sorbic acid, acetic acid and benzoic acid are used to prevent or slow down the growth of microorganisms. However, there is a growing demand for replacing chemical preservatives to ensure food safety and extend the shelf-life.

Packaging materials play an important role in extending the shelf life of food products. They protect the food from deterioration by providing passive protection against the migration of water vapour and gases such as oxygen and carbon dioxide from the atmosphere into the package. Active packaging technology is a branch of packaging science that deals with packages that provide added functionalities in addition to being a passive barrier. Antimicrobial/Self sterilizing Packaging is a type of active packaging technology wherein antimicrobial agents are either blended into or coated on the surface of the packaging materials to extend the shelf life of the product. Metal and metal oxide nanoparticles (NPs) have been reported to be effective against bacteria and other microorganisms (Staron et al., 2021).

Nanoparticles' are solid particles that range from 1 to 100 nanometres in size. Due to their small size, nanoparticles have a high surface area-to-volume ratio than their larger material counterpart. Despite being small, they are able to produce quantum effects. The nanoparticle's properties are mainly determined by its size, shape, and surface. Different nanoparticles are being synthesized using metals like silver, gold,
magnesium, zinc and titanium by chemical, physical and biological methods (Figure 1). Physical methods are expensive and require large amounts of energy just to maintain the optimal conditions of the

process. Even though chemical methods are affordable and high-yielding, they produce harmful by-products and the toxic chemicals in the liquid medium get absorbed on the surface of the nanoparticles synthesized. Biological methods using fungi, enzymes or plant extracts prove to be eco-friendly alternatives to the physical and chemical methods. Because these nanoparticles can be engineered to play a specific role, their use spans various industries. The growing interest in metal NPs led to promising results that make them suitable for producing antimicrobial and antiviral surfaces and coatings.

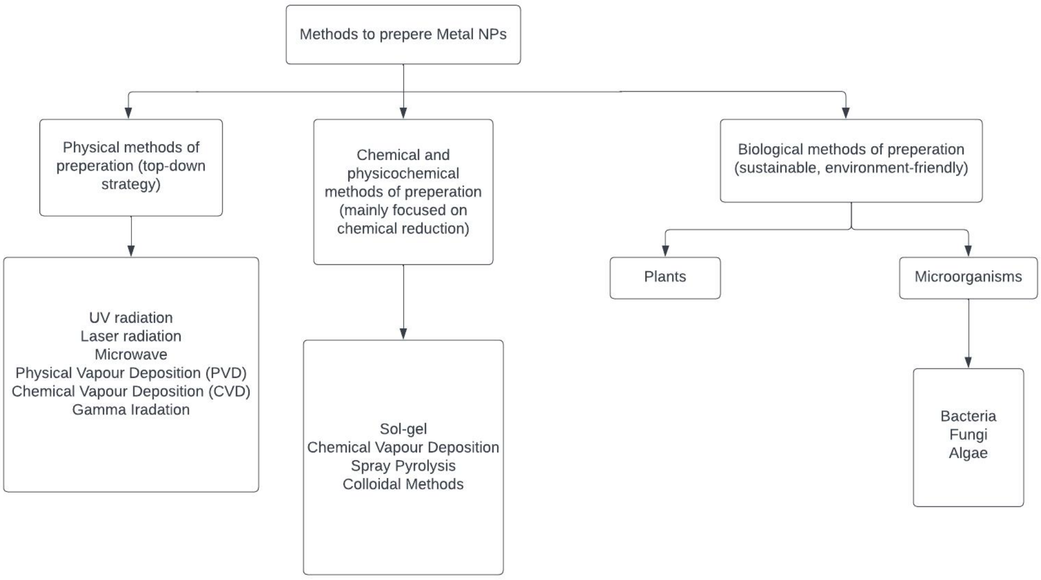

Figure 1: Chemical, physical and biological methods

In this research paper, an attempt has been made to review the mechanisms of metal nanoparticles against microorganisms and their uses in food packaging. Different metal NPs namely Ags, TiO2, MgO, ZnO, Au, FeO, Cu and SnO2 have been analysed.

2. ANTIMICROBIAL MECHANISM OF METAL NANOPARTICLES

Bacteria can be classified as gram-positive and gram-negative bacteria based on the presence or absence of cell wall respectively. The cell wall of gram-positive bacteria is made of peptidoglycans, proteins and multiple layers of murein. However, in gram-negative bacteria, murein is single-layered. The metal NPs mainly affect the structure and function of the cell membrane. This is achieved due to the small size of the metal NPs. Anita Staron et al. (2021) have reported that in interaction with the bacterial cell membrane, the metal NPs affect the membrane integrity, induce oxidative stress, disrupt enzymes, and damage proteins and DNA. The NPs can also get converted into ions and disrupt the metabolic activities of the microorganisms. Moreover, NPs can attack the microorganisms without any direct contact by producing a reactive oxygen species (ROS) which in turn migrates to the surface of the packaging materials and reacts with them. This reaction causes the oxidation of lipids and proteins and the degradation of DNA.

Silver NPs defend against microorganisms by converting them into ions and interacting with enzymes and DNA. At the same time, the gold NPs are able to attach to the cell membranes and reduce the level of ATP and inhibit the binding of tRNA to ribosomes. Zinc NPs in addition to exhibiting similar silver characteristics, also generate ROS on their surface. Copper and Magnesium oxide NPs attack the cell membrane and disrupts the physiological activities of the cell leading to the death of bacteria. Aluminium oxide NPs also creates holes in the cell membrane resulting in the seepage of intracellular materials and the death of microorganism (Mozaffari et al., 2017; Gharpure et al., 2021). The antifungal properties of the metal nanoparticles are listed in Table 1.

Table 1: Antifungal Properties of Metal Nanoparticles

S.No.	Metal NP	Antifungal Effects Caused
1.	Ag	ROS were produced leading to damage to DNA, protein denaturation and leakage of the contentsof the cell. But it was reported that oxidative stress and membrane permeabilization are not the primary reasons for damaging *S. cerevisiae*. In the case of *T. asahii*, these AgNPs caused hyphae deformation and shrinkage, organelle degeneration and leakage of contents in the cytoplasm.
2.	ZnO	Inhibited growth of *C. albicans*
3.	CuO	CuONPs exhibited the same mode of action for bacteria and fungi like *A.niger* and *C. albicans*.
4.	Au	The interaction between AuNPs and Candida sp. inhibited the H+ - ATPase proton pump. This resulted in the inhibition of efflux of H+
5.	Mg doped ZnO	Due to the accumulation of Mg-doped ZnONPs in the cell membranes of *C.albicans* caused cell damage.

3. APPLICATION OF NANOPARTICLES IN PACKAGING

3.1 Silver

Silver is a transition metal. Hence, it has high electrical and thermal conductivity. Also, it is the most reflective metal. This metal has been used as an antibacterial agent in medicines since time immemorial. Silver is spoken for its healing properties. Nanotechnology makes it possible for us to incorporate silver nanoparticles into polymer materials to introduce or enhance antimicrobial properties. This has eliminated the use of silver ions as they form precipitation complexes that inactivate the metal. It has been proved that AgNPs are potentially safer to use and that their antibacterial properties can be enhanced by introducing additives (say, stabilizers) (Wolska et al., 2017).

Self-sterilizing composite polymers, custom packaging and contact surface sterilizers have been successfully produced by reinforcing AgNPs into a host polymer which acts as a matrix that holds the silver nanoparticles (Martínez-Abad et al., 2013; Castro-Mayorga et al., 2017). Also, it is possible to increase the bactericidal effects of the silver NPs by combining 2 metal nanoparticles together. For example, films that contained copper in combination with silver nanoparticles were reported to be more effective than just nanosilver. The nanocomposite films that contained both copper and silver were more effective in the bactericidal action against L. monocytogenes and Salmonella enterica typhimurium (Arfat et al., 2017). When Ag-Cu reinforced films were used as a packaging material for meat (chicken), positive results were obtained. It was observed that the LLDPE/Ag-Cu films showed an appreciative bactericidal activity against pathogens like L. monocytogenes, Salmonella typhimurium and Campylobacter jejuni. It was reported that the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values for AgNPs for Staphylococcus aureus were 0.625 mg/ml (Parvekar et al., 2020).

3.2 Zinc oxide

ZnO-NPs exhibit different morphologies. Also, they are capable of controlling the growth of broad-spectrum bacteria. It can be produced through different methods like Physical Vapour Deposition, Mechanochemical Processing, Hydrothermal Method, solution casting method, etc. (Kimet al., 2022; Channa et al., 2022). Studies prove that ZnONPs act as effective bactericidal agents against microbes like Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, B. megaterium, Pseudomonas vulgaris, and Campylobacter jejuni. E.coli and B. megaterium. However, B.subtilis (endospores) were immune to the bactericidal action of ZnONPs to a certain extent. The ZnONPs were effective only at 36% (20min) and 48% (60min) (Stoimenov et al., 2002). The US FDA considers ZnONPs as GRAS which assures food safety. These NPs are highly toxic to disease-causing pathogens and have a minimal impact on humans. This makes it a promising material to fabricate food packaging with bactericidal properties. Research proves that the mechanical properties, thermal stability and crystallinity of films made of PVA can be increased by reinforcing ZnONPs. The contact angle test proves that the water absorbency and the wettability of the PVA/ZnO nanocomposite films are lower than that of pristine PVA films. On testing the nanocomposite film for its oxygen transmission rate and permeability measurement, it was concluded that the addition of ZnONPs to the PVA did not affect the oxygen barrier properties and OTR. This could be due to the strong wetting between ZnO nanorods and PVA chains. Thus, the constant OTR suggests that ZnO did not introduce porosity and did not alter the structure of PVA. However, the OTR decreased minutely when the ZnO concentration increased (Channa et al., 2022).

3.3 Magnesium oxide

It is versatile in nature. It has been reported that when used along with biopolymers or petroleum-based polymers, desirable results were obtained that encourage active packaging (Ballesteros et al., 2021). It can be produced through different processes like solvent-casting, biosynthesis and colloidal methods (Swaroop et al., 2018; Samadi et al., 2021; Zhang et al., 2020; Abdel-Aziz et al., 2020; Mittag et al., 2019). The mechanical properties (tensile strength and elongation at break) on introducing the NPs have increased. This is because these NPs can block the crack on the surface of the film by absorbing the energy that causes deformation. On the contrary, it was observed that these metal NPs have a negative impact on the film when their composition exceeds 3 wt% because at this concentration, these metal NPs agglomerate with each other which deteriorates the polymer's structure (Zhang et al., 2020). The tensile strength of the PLA/MgO nanocomposite films produced by the solvent-casting method increased by 2 wt% loadings. This was attributed to the uniformity in the dispersion of the MgONPs in the films prepared (Swaroop et al., 2018). The nanocomposite films were observed to be thermally more stable than the pristine films on TGA analysis. The MIC for MgONPs against B. subtilis and E.coli were recorded to be 0.5 and 0.75 respectively. The MBC for MgONPs against B. subtilis and E.coli were recorded to be 1 and 1.5 respectively. The comparatively higher MIC and MBC suggest the fact that E.coli is more resistant to the MgONPs. The MBC value implies that the train is not multi-drug resistant (Bhattacharya et al., 2021).

3.4 Gold

Unlike bulk gold particles, AuNPs have unique properties that make them extremely useful for specific applications. This is solely attributed to their small size and the large surface area-to-volume ratio. Many studies have reported the mechanical properties (tensile strength, elongation at break, etc.) of polymer films have been enhanced because of the reinforcement of gold nanoparticles into the matrix. It was observed that PVA-glyoxal-AuNP films provided better preservation of bananas than the PVA-glyoxal-graphene oxide films with minimal black spot formation for up to five days which could possibly be due to the enhancement of mechanical properties provided by the AuNPs (Chowdhury et al., 2020). PVA/AuNPs films showed that there was a significant increase in the tensile strength, Young's modulus and a decrease in the elongation at break were observed in the films having PVA-GA cross-linking than in PVA films (Chowdhury et al., 2020). On testing for the dynamic viscosity of the nanocomposite films, the result suggested a decrease in the dynamic viscosity of the chitosan/ aminopropyl silane/AuNPs films owing to the homogeneity and the miscibility of the film. When tested for antimicrobial activity, these chitosan/aminopropyl silane/AuNPs films showed antibacterial activity against Salmonella bacteria. These bacteria cause Salmonellosis and other food-borne diseases. This is attributed to the inherent antibacterial activity and the positive charges present in chitosan, APTMS and AuNPs. The composite film interacted with the bacterial cell wall to damage it and eventually lead to the lysis of the bacterial cell (Virgili et al., 2021). The Minimum Inhibitory Concentration (MIC) for the AuNPs that were 7–34 nm in size were 2.93 µg/mL, 7.56 µg/mL, 3.92 µg/mL, and 3.15 µg/mL for E. coli, B. subtilis, S. aureus, and K.pneumonia, respectively. While that for AuNPs of size 20-40 nm were 2.96 µg/mL, 8.61 µg/mL, 3.98 µg/mL and 3.3 µg/mL for E. coli, B. subtilis, S. aureus, and K. pneumonia, respectively (Shamaila et al., 2016).

3.5 Titanium dioxide

TiO2 NPs are well-known, easily available and low in toxicity. It has gained much attention for its antimicrobial properties. It proved to be suitable for producing nano-blend food packaging films without deteriorating food safety when it was reinforced with PLA films. There was an increase in the tensile strength, elastic modulus and stiffness in the PLA/TiO2 and PLA/TiO2/Ag films. The cross-section showed that the PVA films became rougher with the introduction of the NPs (Li et al., 2017). On studying the gelatin biopolymer films reinforced with 1% (w/w) TiO2 NPs and saffron extract 2% (w/w), it was observed that the addition of saffron extract and TiO2 NPs increased the thickness and mechanical properties of the films and that the moisture content, water vapour permeability and solubility were reduced (Azimi-salim et al., 2022). It was studied that the pristine PLA films showed no antimicrobial activity on E.coli and Listeria monocytogenes. The films with a certain concentration of TiO2 NPs showed significantly reduced growth of the bacteria in the culture plate. This activity increased with the increase in the concentration of the NPs (Li et al., 2017). While for LDPE/Ag/TiO2 and LDPE/Ag + Cu/TiO2 nanocomposite films, it was observed that the maximum antibacterial protection to Tilapia was provided by the films containing 2.5% silver, 2.5% copper and 5% titanium dioxide NPs (Efatian et l., 2021). Thus, TiO2 NPs prove to be a promising material for producing nano-blend packaging films without deteriorating food safety (Li et al., 2017).

3.6 Tin dioxide, copper-based and ferrous oxide NPs

These nanoparticles have been already employed in multiple fields due to their electrical conductivity, ease of availability and antimicrobial properties. It was observed that the electrical conductivity increased by increasing the concentration of Indium Tin Oxide NPs (ITONPs) in the PANI films as the ITONPs filled the gaps present in the case of the pristine PANI (polyaniline on carbon black) films. There was no reaction happening between the metal MPs and the polymer. However, the NPs interact with each other. The disorderly arrangement of the polymer film increases when the NPs are introduced because the NPs widen the diameter of the spaces present in the matrix of the film. In other words, this suggests that NPs increase the crystallinity in the film. The surface roughness of the film containing 16 wt% of the NPs is 0.24 µm (Al-Bataineh et al., 2022). It was observed that coating FeONPs with, materials like polyvinylpyrrolidone (PVP), or polyethene glycol (PEG) increased the antiviral properties (Kumar et al., 2014). On analysis of CuNPs-C-PLA nanocomposite films, good antibacterial action was exhibited by the NPs against Pseudomonas spp. (Longano et al., 2012).

4. CONCLUSION

Self-sterilizing packaging is widely used in areas where pathogenic microbes are present. This seems to be a clever solution to any pandemic breakdown. Almost all metal NPs follow a similar mechanism of producing ROS in order to cause cell death for both bacteria and fungi. AgNPs are extensively used in commercial packaging and have increased the shelf-life of food products. Works of literature on the AuNPs in this subject are very few which might be because of its high cost. MgONPs exhibited practically significant properties when reinforced into a PLA film. Being safe and nontoxic, ZnONPs are being used in the packaging industry currently. Copper-reinforced cellulose nanocomposite packaging materials that were developed showed good biocidal activity against E.coli. Titanium Dioxide, Tin(IV) Oxide and Ferrous Oxide NPs prove to be good choices for producing a self-sterilizing packaging material.

5. CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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