



A Critical Review on The Integration of Metal Nanoparticles in Biopolymers: An Alternative for Active and Sustainable Food Packaging

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Abstract

The use of plastic polymers in food packaging causes serious environmental and health problems and as a result, natural biopolymers (NBPs) are being developed. Although NBPs have several shortcomings as a packaging material, these can be overcome with the help of nanotechnology. In this context, this review will report on the main findings about the effect of the integration of metal nanoparticles (MNPs) on the characteristics of NBPs. A systematic review was carried out using PRISMA methodology to select relevant studies from the last 5 years. According to the analysis performed, MNPs provide NBPs with a broad spectrum against bacteria, fungi and even viruses of interest. MNPs have also been shown to improve the physical, mechanical, optical, antioxidant and barrier characteristics of NBPs. MNPs are used at low concentrations (generally 0.5 to 5%) and this avoids their potential toxicity. MNPs are shown to be efficient materials to obtain bionanocomposites suitable for active food packaging. Studies focusing on the control of the antimicrobial effect of MNPs on desirable microorganisms are suggested. In addition, further studies on the evaluation of the potential toxicity of MNPs are needed to ensure food quality and safety.



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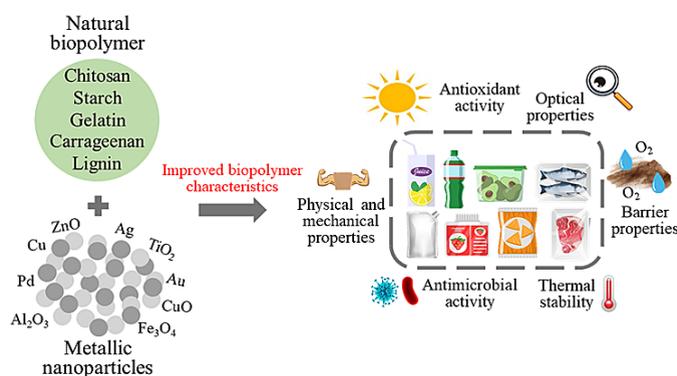


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Graphical Abstract



Introduction

Nanotechnology is having increasing progress and efficiency in different scientific fields,¹ such as energy, electronics,² water treatment,³ chemistry, biology,⁴ materials engineering, pharmaceuticals, biotechnology,⁵ medicine and agriculture.⁶ Specifically, it is revolutionizing the food industry worldwide, due to the multiple applications it offers.⁷ Nanoencapsulation,^{8,9} the use of nanomaterials and nanosensors in the development of active and/or smart food packaging are highlighted.

Emphasizing the food packaging, it is a dynamic market which exceeded 300 billion dollars in 2019 with an approximate growth of 5.2% per year.¹⁰ This is due to its role in food quality and safety.¹¹ With regard to the manufacture of packaging, demand for nanocomposites is increasing substantially.² In the European market in 2015, more than \$2.5 million was generated, with a revenue forecast of approximately \$9 million by 2022.¹²

It is important to mention that a food packaging must be made of low-cost materials with adequate hardness, flexibility, lightness, strength and inertness, among other properties, in addition to being easily moldable. Polyethylene and polypropylene meet the above requirements, but since they are plastic polymers, they take more than 100 years to degrade and cannot be reused.¹³ Considering that the world production of plastics exceeded 350 million tons in 2015,¹⁴ they represent a high environmental pollution.

To solve the aforementioned problem, avoiding the accumulation of synthetic materials and satisfying the global demand for sustainable, safe

and quality products, biodegradable materials have been used. They do not represent any risk to the environment or health, they are reusable and easy to dispose of.¹⁵ For their production, natural compounds obtained from renewable materials of plant, animal and microbiological origin are used. However, despite the benefits of natural biopolymers (NBPs) manufacturing, they do not have optimal physical, mechanical and barrier characteristics. In this context, with the help of nanotechnology, inorganic and non-toxic nanomaterials are currently being used, in particular metal nanoparticles (MNPs).¹⁶ MNPs are biocompatible, therefore they can be incorporated into NBPs, forming a hybrid system, an ideal bionanocomposite to replace traditional packaging. MNPs improve the properties of NBPs, contributing to active, novel and efficient packaging.^{17,18} A glycerol plasticized-pea starch film exhibited poor mechanical and barrier characteristics. On the other hand, by incorporating a loading (5%) of ZnO nanoparticles stabilized with carboxymethyl cellulose (CMC), the tensile strength increased by 9.81 MPa, and 42.2% of elongation at break and $11.2 \times 10^{-7} \text{ g} \cdot \text{m}^{-1} \cdot \text{h}^{-1} \cdot \text{Pa}^{-1}$ of WVP were reduced. In addition, the film had a higher UV-visible absorption.¹⁹ As can be seen, another advantage of MNPs is that they are used in small concentrations, avoiding modifying the polymeric matrix or causing negative effects on food quality.

Considering the importance of constant innovation of packaging, MNPs have attracted the interest of many researchers.^{20,21} However, their application in the food sector has not yet been widely explored. In order to fill a gap and updated the state of the art of the mentioned topic, this study will highlight

the most recent and significant findings on the improvement of antimicrobial activity and other properties of NBPs with the incorporation of MNPs (NBP-MNPs). This will help and encourage scientists to be more interested in this nanotechnology that is promising in obtaining more efficient active food packaging. Some limitations/risks related to the use of MNPs will also be detailed, such as their potential toxicity, which is necessary to understand in order to avoid possible risks.

Methodology

Systematic Search Method

The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement was followed.

Questions of Interest

In order to solve the research problem, the following questions were stipulated: What types of NBPs and MNPs are most commonly used in food packaging and in what proportion? Which microorganisms have been efficiently inhibited in foods packaged with NBP-MNPs? What other NBP characteristics are improved by the incorporation of MNPs?

Literature Sources and Search Strategy

The Scopus and Science Direct databases were consulted on September 30, 2021. The following string was used: TITLE-ABS-KEY (Metallic OR Inorganic) AND (Nanoparticles OR Nanocomposites OR Nanomaterials) AND (Packaging OR "Food Packaging" OR "Food Container").

Eligibility Criteria

Only research articles in English and published in the period 2017-2021 were considered. Mendeley was used to manage and eliminate duplicate articles extracted from the two databases.

In the screening stage, the title, abstract and key words of each article were examined to exclude those that did not relate to the study topic. Finally, a full-text analysis was performed to ensure the appropriateness of the articles. Additionally, the snowballing technique was applied, which consists of examining the references of the selected articles and including those relevant to the objective of this study.

Analysis of Extracted Studies

Results of The Prisma Statement

A detailed summary of the process executed is shown in Figure 1, which generated the extraction of 14 documents ready for analysis. From these studies we extracted the data needed to answer the research questions.

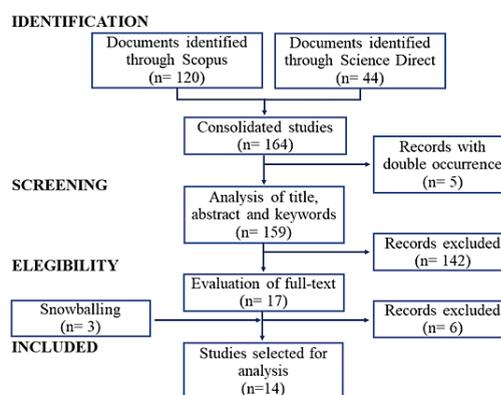


Fig. 1: Flow chart for the selection of studies using the PRISMA methodology.

Antimicrobial Potential of Metallic Nanoparticles

The use of biomaterials (BPs) in packaging is being widely studied. To improve them, multiple MNPs and their oxides have started to be integrated in recent years. The most important are silver (Ag),¹⁶ gold (Au), platinum (Pt),⁹ zinc (Zn), zinc oxide (ZnO),¹⁵ titanium dioxide (TiO₂),²² copper (Cu), copper oxide (CuO), nickel oxide (NiO),²⁰ cerium dioxide (CeO₂),³ calcium oxide (CaO), magnesium oxide (MgO)¹ and cadmium (Cd).²³ Magnetite (Fe₃O₄) and maghemite (Fe₂O₃), called super paramagnetic, are also used.²⁰ Their use of MNPs has had optimal results individually, but the combination of several MNPs is mainly recommended to obtain synergistic effects.²⁴ This can enhance the antimicrobial activity up to 8 times.²⁵ As shown in Table 1, starch, gelatin, lignin, CMC and chitosan, in equal proportion (in 2 of the 14 selected studies), were the most used to develop NBPs. The proportion of MNPs used was low (from 0.5 to 5%), but did not affect their performance, which is due to their high surface area/volume ratio.^{2,9} Likewise, ZnO nanoparticles were the most used in (9 of 14 studies).

According to multiple studies, MNPs have a broad antimicrobial spectrum. Using ZnO and

CuO nanoparticles, inhibition of *Escherichia coli* (4.3 and 4.2 mm), *Staphylococcus aureus* (2.1 and 2.3 mm), *Staphylococcus epidermidis* (2 and 2.3 mm) and *Listeria monocytogenes* (1.8 and 2.1 mm) were reported.²⁶ In another study, Cu (5%), Pd (5%) and Ag (5%) nanoparticles had a high effect against *E. coli* (32.93, 30.13 and 24.87 mm) and *L. monocytogenes* (34.83, 31.90 and 26.93 mm).⁶ The inhibitory effect of a 60 µg Ag nanoparticles/mL solution against *S. aureus* (21.10 mm), *E. coli* (19.13 mm), *Bacillus subtilis* (18.07 mm) and *Pseudomonas aeruginosa* (18.07 mm) was reported.²⁷ Likewise, 200 µl of 0.1% ZnO nanoparticles solution was efficient against *S. aureus* (17 mm), *B. subtilis* (10 mm) and *E. coli* (8 mm).²⁸

Considering the above, in addition to what is detailed in Table 1, it can be seen that MNPs have a greater effect on Gram-negative (G-) bacteria

than on Gram-positive (G+) bacteria. This is due to the structure of their cell walls.⁴⁷ G+ bacteria have a thick peptidoglycan layer, but being a single layer, it is more permeable and easily allows the binding of MNPs.²⁰ On the other hand, G- bacteria have a thinner peptidoglycan layer, but they also have an outer phospholipid layer with lipopolysaccharides, providing them with greater protection.¹³ However, due to the composition of their external membrane, G- bacteria have a greater negative surface charge, which causes a greater attraction and electrostatic interaction with cationic MNPs.²⁹

Regarding antifungal activity, super paramagnetic iron oxide and Ag nanoparticles at concentrations of 25 ppm showed effect against *Fusarium solani* and *Aspergillus niger*.¹⁸ Ag (100 ppm) nanoparticles inhibited the concentration of *Corynespora cassiicola* by 85% and completely eliminated *Alternaria solani* and *Fusarium spp.*³⁰

Table 1: Findings on the antimicrobial activity of NBP-MNPs evaluated in selected studies.

NBPs / %	MNPs / %	PMM	Microorganisms evaluated	Microbial reduction	References
Bacterial nanocellulose / N.S.	Cu / 5% Ag / 5% Pd / 5%	Off-site incorporation	<i>E. coli</i> O157:H7 and <i>L. monocytogenes</i>	30.00 and 24.13 mm at 24 h 31.73 and 26.40 mm at 24 h 22.77 and 29.27 mm at 24 h	6
Soy protein isolate / 6%. CMC / 5%.	ZnO / 0.2%.	SC	<i>A. niger</i>	≈1.7cm against <i>A. niger</i>	11
	Ag / 2% ZnO / 2% CuO / 2%	SC	<i>E. coli</i> and <i>S. aureus</i>	0.18 and 0.06 mm at 24 h 0.02 and 0.02 mm at 24 h 0.01 and 0.00 mm at 24 h	24
Starch / N.S.	Ag-ZnO-CuO / 0.667%	SC	<i>E. coli</i> and <i>S. aureus</i>	93.99% and 93.31% at 24 h	25
Fish skin gelatin / 3%.	Ag-Cu / 4%	SC	<i>L. monocytogenes</i> and <i>S. typhimurium</i>	3 and 7 log CFU/ml after 7 days	28
Gelatin-starch / 8.8%	ZnO / 1%	SC	<i>E. coli</i> and <i>S. aureus</i>	85.38 and 67.28 mm at 18 h	31
Nanolignin-PLLA / N.S.	Ag / 1%	SC	<i>E. coli</i> and <i>L. monocytogenes</i>	100% at 3 h and 100% at 6 h	32
CMC-chitosan / N.S.	ZnO / 2%.	SC	<i>A. niger</i>	40.13%	33
PHBV / 10%. Chitosan nanofibers / 2%	ZnO / 3%. CuO / 1%	Electrospinning Chemical precipitation	<i>E. coli</i> and <i>S. aureus</i> <i>E. coli</i> , <i>L. monocytogenes</i> and <i>P. aeruginosa</i>	≈ 3.2 and 3.3 m mat 24 h 18,17, 22.21 and 27.19 mm at 24 h	34 35
Galactomannan / 1%.	ZnO / 0.2%.	Ultrasonic dispersion and SC	<i>E. coli</i> and <i>B. subtilis</i>	99% and 99% at 24 h	36
Gelatin-PVA / N.S.	TiO ₂ -ZnO / 1%.	SC	<i>E. coli</i> , <i>L. monocytogenes</i> , <i>S. aureus</i> and <i>Pseudomonas fluorescens</i>	11.12, 11.75, 11.41 and 12.63 mm at 24 h	37

PLA-nanolignin / 10%.	Ag ₂ O/ 0.5% WO ₃ / 0.5% TiO ₂ / 0.5% Fe ₂ O ₃ / 0.5% ZnFe ₂ O ₄ / 0.5%	SC	<i>E. coli</i> and <i>S. aureus</i>	50, ≈ 42, ≈ 40 and ≈ 15% against <i>S. aureus</i> at 6 h. Only TiO ₂ (20%) and ZnFe ₂ O ₄ (5%) showed effect against <i>E. coli</i> at 6 h	38
Carrageenan /2%.	ZnO-CuO /0.5%	SC	<i>E. coli</i> and <i>L. monocytogenes</i>	100% at 6 h and ≈ 50% at 12 h	39

N.S.: not specified, PMM: polymer manufacturing method, SC: solution casting method, PLLA: poly(L-lactide), PHBV: poly (3-hydroxybutyrate-co -3-hydroxyvalerate), PVA: poly(vinyl alcohol), PLA: polylactic acid.

In another study, Ag nanoparticles reduced mycelium of *Alternaria alternata* (22%) and *Pyricularia oryzae* (68%). Similar results were obtained when CuO nanoparticles was used, but ZnO nanoparticles showed no significant effect. In addition, none of the MNPs had any effect against *Sclerotinia sclerotium*.²³ Likewise, 40.13% inhibition of *A. niger* was reported using CMC-chitosan-oleic acid-ZnO nanoparticles (2%).³³

Regarding evaluation in food, in an investigation, the polymer chitosan-ZnO nanoparticles (1%) was evaluated. The concentration of *E. coli* O157:H7 in white cheese in brine was reduced during storage for 28 days at 4 °C (from 4.44 to 1.57 log colony-forming unit (CFU)/g) and 10 °C (3.71 to 2.18 log CFU/g), respectively.⁴⁰ In another study and using a polymer based on thermoplastic starch-PVA-Ag nanoparticles, the load of psychrotrophic and mesophilic microorganisms was reduced from 8.16 to 7.70 log CFU/g and 8.62 to 7.87 log CFU/g, respectively, in ham slices after 7 days.⁴¹

Studies on the antiviral effect have also been reported.²⁰ This is important mainly due to the current COVID-19 pandemic, so there is a need to develop effective antiviral methods. Different MNPs such as Ag 16 have been shown to externally and internally influence the person, preventing virus entry and replication.¹³ Apolyethylene-carvacrol-ZnO nanoparticles and polyethylene-geraniol- ZnO nanoparticles coatings reduced the concentration of phi 6 phages by 1 log after 24 h. Phi 6 bacteriophages are being studied extensively as substitute for SARS-CoV-2. It was determined that the antiviral potential of ZnO nanoparticles was enhanced by the anti-SARS-CoV-2 activity of carvacrol and eugenol, showing its effect on the main structural protein of the virus, the spike

protein.⁴² Similarly, polyvinyl chloride-SiO₂-Ag nanoparticles reduced by 81.58 and 99.99% the viability of SARS-CoV-2 by contact at 3 and 15 min, respectively.⁴³ The key to the activity against SARS-CoV-2 is the reactive oxygen species (ROS) generated by the Ag nanoparticles and SiO₂, in addition to the high antioxidant power of the metallic base.⁴⁴ These properties are suitable for causing irreversible damage to structural and non-structural proteins in SARS-CoV-2.

The antimicrobial effect of MNPs is influenced by their synthesis method, shape, size and type.⁴⁵ The different mechanisms of action (Figure 2) are through their metal ions¹³ and ROS such as superoxide anion (O₂⁻), singlet oxygen (O₂¹), hydroxyl radical (OH) and hydrogen peroxide (H₂O₂). First, MNPs adhere to the microbial surface and the strong toxicity and oxidative activity of the ROS produced counteract the microbial antioxidant defense.²⁹ Subsequently, ROS together with metal ions begin to damage cellular structures, inducing lipid peroxidation,⁴⁶ membrane permeabilization and disintegration, damage to DNA, ribosomes, enzymes,⁴⁷ protein functional groups such as carboxyl (-COOH), amino (-NH) and thiol (-SH).⁴⁸ In addition, the transmembrane electron transport chain and metabolic pathways of morphological and physiological importance are blocked.^{20,28,34} This causes alterations in vital functions such as cellular respiration, leading to inhibition of microbial growth and ultimately cell death.

Effect of Metallic Nanoparticles on other Characteristics of Biopolymer

With the integration of MNPs into NBPs, an active and complete package is achieved (Figure 3). Table 2 shows the findings of selected studies that have evaluated other characteristics of NBP-MNPs

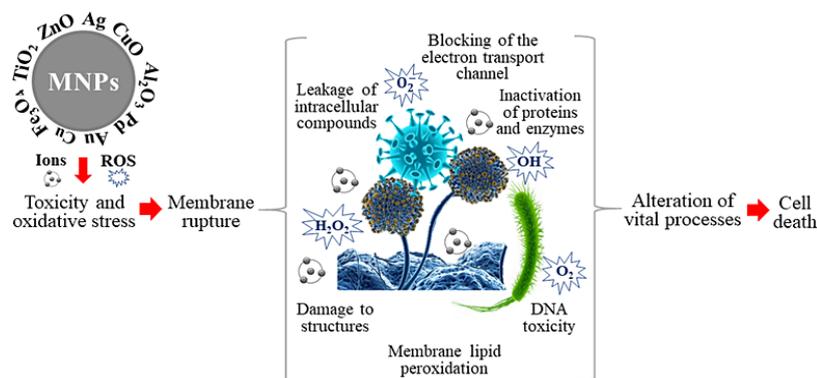


Fig. 2: Mechanism of antimicrobial action of MNPs.

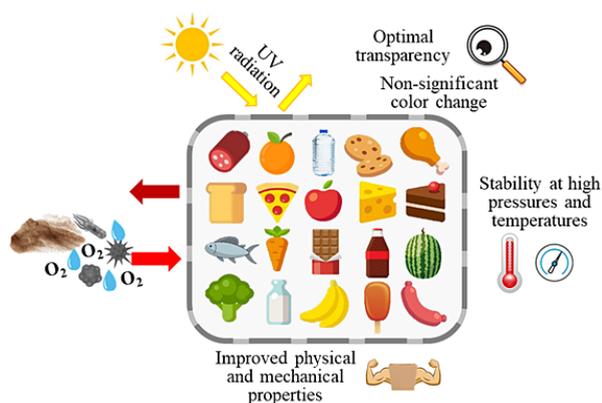


Fig.3: Enhancement of NBPs functionalities through MNP Sintegration.

Physical Properties

In an investigation, the thickness of a film of chitosan-essential oil of *Melissa officinalis* was increased by integrating ZnO nanoparticles due to their influence on the increase of the film density.⁴⁹

Similar results were shown in the evaluation of a carboxy methyl chitosan (CMCH)-ZnO nanoparticles film ($\approx 0.25-2.5\%$), 50 in a skin gelatin-Ag and Cu nanoparticles film (1-4%),²⁸ and in a gelatin-starch-Zn nanoparticles film(12.5%).³¹

Table 2: Summary of the evaluation of the effect of MNPs on other characteristics of NBPs in selected studies.

NBPs / MNPs	Main Findings	References
CMC / ZnO, CuO and Ag	Increase of UV absorption rate. Reduction of WVP. Improvement of YM, TS and EB	24
Starch / Ag-ZnO-CuO	Reduction of WS, WVP and EB. Increase of TS and YM. Optimum UV and visible absorbance	25
Fish skin gelatin / Ag-Cu	Increase of thickness, TS, a* and b* value, ΔE, transparency and TDT. Reduction of EB and L* value. Darker color	28
Gelatin-starch / ZnO	Increased of thickness, TS and melting temperature. Reduction of EB, WVP and WS	31

Nanolignin- PLLA / Ag	Increase of a* and b* value, ΔE, thickness, TS and YM. Reduction of L* value, EB and WVP	32
CMC - chitosan / ZnO	Reduction of TS, YM, L* and a* value. Increase of b* value, ΔE, chroma value, EB and contact angle	33
PHBV/ZnO	Reduction of L*, a* and b* value and EB. Increase of transparency, TS, toughness and TDT	34
Galactomannan/ ZnO	Increase of UVA and UVB absorption, TS, YM, contact angle and TDT. Reduction of OP and WVP.	36
Gelatin-PVA/ TiO ₂ -ZnO	Reduction of transparency, WVP, OP and EB. Increase of TS, YM and thickness.	37
PLLA-nanolignin / Ag ₂ O, TiO ₂ , WO ₃ , Fe ₂ O ₃ and ZnFe ₂ O ₄	High antioxidant activity. Reduction of thermal resistance in the addition of MNPs in PLA, but it was stabilized with the presence of lignin. UV and visible transmittance was also significantly reduced. Darker color	38
Carrageenan/ ZnO-CuO	Increase of hardness, strength, thickness, EB and yield. Reduction of swelling, TS and YM. Equal TD with residue increase. Darker color due to CuO	39
Soy protein isolate / ZnO	Reduction of L* value, whiteness index, WVP and OTR. Increase of a* and b* value, ΔE, transparency, EB and TS Good barrier against UV and visible light	50

L*(lightness), a*(red/green) and b*(yellow/blue) in CIELAB color space, ΔE: total color difference, UVA and UVB: types of UV radiation, WS: water solubility, WVP: water vapor permeability; OP: oxygen permeability, OTR: oxygen transmission rate, TS: tensile strength, EB: elongation at break, YM: Young's modulus, TDT: thermal degradation temperature.

The morphology of a CMCH-MgO nanoparticles (0.5 and 1%) film was evaluated by scanning electron microscopy. It was determined that the addition of nanoparticles caused the film to be thicker and heterogeneous. Furthermore, according to the evaluation of the intermolecular interaction by Fourier transform infrared spectroscopy (FT-IR), the addition of MgO nanoparticles generated the reduction of the -OH band (from 3432 to 3425 cm⁻¹) and C – O – C bands (from 1070 to 1045 cm⁻¹). This indicates that the nanoparticles caused chemical and physical changes in the film such as decreased WS.⁵¹ Similar results were shown when evaluating a CMC-chitosan-sodium alginate-ZnO nanoparticles (≈ 0.25-2.5%) film⁵⁰ and in a chitosan-essential oil of *Melissa officinalis*-ZnO nanoparticles film (0.01 y 0.03%).⁴⁹ In another study, Ag-Cu nanoparticles (1-4%) generated roughness in a skin gelatin film, despite being uniformly distributed in it. In addition, the peak of the NH (from 3280 to 3270 cm⁻¹) and C – H (from 2927 to 2915 cm⁻¹) stretching bands was reduced by the formation of hydrogen bonds.²⁸

Ag nanoparticles aggregates and spots were visualized in montmorillonite-boiled rice starch-Ag nanoparticles film (0.1%). In addition, the C - H group band was shifted to a lower wavenumber (from 2941 to 2916 cm⁻¹) and there was also interaction between the -OH group of the compounds due to changes in the 3300-3000 region cm⁻¹.⁵² Ag nanoparticles (0.1-0.3%) caused roughness in a chitosan film, in addition to being distributed unevenly in the matrix due to their aggregation and increased viscosity. It was also determined that there was a reduction of the wavenumber from 3290 to 3278 cm⁻¹, indicating the interaction and binding between the Ag nanoparticles and the nitrogen and oxygen atoms of the chitosan.⁵³

As a particular case, Ag nanoparticles (1 mM) were uniformly dispersed in the Agar-banana powder film, but no physical or chemical changes were detected in the film.⁵⁴ The same results were shown by evaluating a CMC-PVA-CuO nanoparticles film (0.3-0.9%),⁵⁵ in a cellulose-Cu nanoparticles (5-250 mM) film,⁵⁶ and a carrageenan-CuO (0.5%) and ZnO (0.5%) nanoparticles film.³⁹

It is also essential to avoid packaging degradation at different temperature conditions during and after processing, as this could affect its properties and those of the food. Regarding thermal properties, PHB film and Pd nanoparticles (1%) had a mass loss of 5% at 224 °C and degraded completely at 368 °C. In addition, the PHB-Pd nanoparticles film had a residual mass of 3.17% at 500 °C, while the residual mass of the PHB film was 3.08%.⁵⁷ Similar results were shown in the manufacture of PHBV-ZnO nanoparticles (0.75-2.25%) film whose residual mass at 700 °C was 4.25% compared to 2.10% for PHBV film.³⁴ A CMCH film and a CMCH-MgO nanoparticles film (0-5 and 1%) lost 60% of their weight at a thermal degradation of 289 and 536 °C, respectively.⁵¹ The addition of AgNO₃ nanoparticles (0.06-32%) into a starch-PVA film increased the residual mass at 600 °C from 13.7 to 25%.⁵⁸ Likewise, the incorporation of Ag-Cu nanoparticles (1-4%) increased the residual mass of a skin gelatin film from 21.58 to 28.49% at 600°C.²⁸

In another study, ZnO nanoparticles (0.75-2.25%) increased the thermal stability of a PHBV film during heating from 25 to 700 °C due to the catalytic properties and high thermal conductivity of ZnO nanoparticles.³⁴ Otherwise, it was determined that the plasticizing properties of ZnO nanoparticles (0.5-2%) influenced the reduction of the thermal stability of a chitosan-CMC-oleic acid film. This effect was also attributed to some characteristics of oleic acid.³³

Mechanical Properties

In a study, TS increased in fish skin gelatin film when the concentration of the combination of incorporated Ag and Cu nanoparticles was 0.5 to 2%, but at 4%, the TS decreased. The authors determined that this deficiency was due to the supersaturation of nanoparticles in the film.²⁸ Ag nanoparticles increased the peel strength of a carrageenan-laponite film due to the surface roughness caused.⁵⁹ Likewise, an increase in hardness (737-989 g/cm²) and strength (743-975 g/cm²) was shown when incorporating CuO and ZnO nanoparticles separately (1% each) and in combination (0.5% each) into a carrageenan film.³⁹

The integration of Pd nanoparticles (1%) to PHB film increased YM (151 MPa), TS (4.7 MPa) EB (0.2%) and toughness value (0.1 mJ/m³), which showed the correct dispersion of Pd nanoparticles in the matrix.⁵⁷ The CMC-chitosan-chitosan-sodium alginate-ZnO nanoparticles film (\approx 2.5%) showed an increase in TS (8.13 MPa) due to internal polymer friction, and a reduction in EB (2.55%) due to the formation of new hydrogen bonds in the film.⁵⁰ TS (4.76-13.37 MPa) and EB (1.19-2.67%) values were increased in anchitosan-Ag nanoparticles film (0.1-0.3%) due to the intermolecular formation of ester bonds.⁵³ In a carrageenan-tea tree oil-zinc sulfide nanoparticles (ZnS, 2%) film, high mechanical strength was shown due to the interfacial interaction between the components and the high surface area of the nanoparticles.⁶⁰ Furthermore, the incorporation of Ag, CuO and ZnO nanoparticles separately (2% each) or in combination (0.667% each) into a starch film increased TS (0.5-3.83%) and YM (6.7-17.64 MPa) and reduced EB (21.33-42.33%) due to the optimal intermolecular interaction in the matrix.²⁵ The crystallization of a sodium alginate-zein-betanine alginate film was increased due to the incorporation of TiO₂ nanoparticles (0.5%) caused an increase in TS (10.61 MPa), EB (29.75%) and YM (17.92 MPa).⁶¹ In contrast, the addition of MgO nanoparticles (0-5 and 1%) reduced the TS (\approx 0.5-4 MPa) in a CMCH film due to weak adhesion by the nanoparticles.⁵¹

Barrier Properties

In an investigation, the integration of SiO₂-ZnO nanoparticles into chitosan-PVA packaging allowed for reduced OTR and water vapor transmission rate (WVTR).⁶² A purple corn extract-chitosan-Ag nanoparticles film also showed a significant reduction of WVP and scattering/blocking the passage of light.⁶³

ZnO nanoparticles (\approx 2.5%) covered the empty spaces in the structure of CMC-chitosan-sodium alginate film⁵⁰ and chitosan-essential oil of *Melissa officinalis* film,⁴⁹ causing a significant reduction in WVP. Incorporation of Ag, CuO and ZnO nanoparticles separately (0.667% each) and in combination (2%) reduced WVP (\approx 0.01-0.9 x 10⁵ g/m.h.Pa) in a CMC film. This is due to

physical changes in the film, which caused more difficult pathways for the movement of oxygen and water vapor.²⁴ WVTR was also reduced (≈ 0.2 - 1.7 g.mm/m².day) in a PLLA film by the incorporation of TiO₂, Fe₂O₃, SiO₂ and Al₂O₃ nanoparticles separately (1% each), but OP was only slightly reduced (≈ 0.02 - 0.05 sweep) with the incorporation of SiO₂ and TiO₂. The authors did not justify these results, but suggest that it could be due to the strong competition between oxygen and water vapor to cover the empty spaces in the matrix. In this case, only the nanoparticles that show higher dispersion are able to immobilize the passage of water molecules and also oxygen molecules.⁶⁴ Likewise, the incorporation of Ag, CuO and ZnO nanoparticles separately (0.667% each) or in combination (2%) into a starch film reduced its WS (1.39-8.07%) and WVP (0.55 - 3.91×10^{-7} g/mh.Pa). In this case, the reason was the influence of the nanoparticles on the reduction of OH- bonds of the starch, which generated a hydrophobic film.²⁵ Due to the hydrophobicity and low permeability of CuO nanoparticles (1-2%), their incorporation reduced the WVP (19.4 - 34.5×10^{-8} g.m/m².Pa.h) of a CMC-kefir film.⁶⁵ Similar results were shown when evaluating a carrageenan-agar-ZnS nanoparticles film.⁶⁰

Interestingly, despite the hydrophobic nature of TiO₂, its incorporation at low concentrations (0.5 and 1%) reduced the WVP value (from 8.4 to 6.6×10^{-10} g.m/m². Pa.s) in a gelatin-grapefruit seed extract film. However, at high TiO₂ concentrations (3 and 5%) the WVP value was 27 and 28.8×10^{-7} g.m/m².Pa.h. This occurred due to the high aggregation of the nanoparticles and subsequent separation of the gelatin chains, which generated more empty spaces in the film and facilitated the passage of water vapor.⁶⁶ Also, the incorporation of ZnO nanoparticles (0.2-5%) into a PLA film caused its depolymerization. This caused a poor OP (from 0.27 to 0.67×10^{-17} m³.mm⁻².s⁻¹.Pa⁻¹), compared to the value of the control sample (0.6×10^{-17} m³.mm⁻².s⁻¹.Pa⁻¹).⁶⁷

MNPs also have the ability to eliminate/absorb ethylene released by fruits and vegetables in the package, which gives them a longer freshness and shelf life.²⁴ The chitosan-TiO₂ nanoparticles (0.25-2%) film showed excellent ethylene removal by photodegradation, the effect of which increased

with TiO₂ concentration. The film also showed a reduction of WVP due to the water insolubility of TiO₂, which blocks the passage of water vapor.⁶⁸

Optical Properties

Zn nanoparticles (0.2%) caused a reduction in brightness and produced a more greenish and yellowish appearance to a film of soy protein isolate-cinnamaldehyde by reducing the L* value (from 85.24 to 79.67) and increasing the a* (from -0.12 to 0.51) and b* value (from 9.29 to 22.33), respectively. The film became darker due to surface plasmon resonance characteristic of nanoparticles.¹¹ The incorporation of TiO₂ (0.5-5 wt% of gelatin) into a gelatin-grapefruit seed extract film caused a decrease in brightness (L* value, from 91.2 to 82.7) and an increase in reddish (a* value, from -0.6 to 1.2) and yellowish (b* value, from 5.5 to 38.4) tones.⁶⁶

Unlike ZnO nanoparticles (1%), CuO nanoparticles (1%) are dark and this caused opacity in the poly-ε-caprolactone-terephthalic acid film.⁶⁹ The same result was shown in other study where cellulose films with Cu nanoparticles (5-250 mM) were fabricated. The films changed from light brown to darker tones, proportionally to the concentration of Cu nanoparticles.⁵⁶ In another study, starch-PVA films with the integration of AgNO₃ nanoparticles (0.06-32%) turned from colorless to light brown and even dark brown, which was attributed to the reduction of silver.⁵⁸ Similarly, the opacity of a film of chitosan and essential oil of *Melissa officinalis* increased with the integration of ZnO nanoparticles ($\approx 2.5\%$).⁴⁹ APVA-montmorillonite-boiled rice starch film lost its transparency and turned brown by the addition of Ag nanoparticles (0.1%).⁵² The PHBV-ZnO nanoparticles film (0.75-2.25%) had higher opacity than the PHBV film and with a ΔE value of 1.83, determining that the nanoparticles did not significantly affect film color.³⁴

Regarding food evaluation, in an investigation, the quinoa starch film with Au nanoparticles (2.5 and 5%) had higher absorbance values in the UV-visible region (500 nm), which means lower transparency/higher opacity. The same occurred in the UV region (210 nm), determining that the film is an adequate defense against UV rays and their effects on, for example, lipid oxidation of foods.⁷⁰

It was reported that MgO nanoparticles (0.5 and 1%) increased the UV absorption capacity of the CMCH film.⁵¹ In an Agar-banana powder film, the addition of Ag nanoparticles (1 mM) reduced the light transmittance at 280 nm, but not at 660 nm.⁵⁴ The light absorption and light scattering ability of ZnO nanoparticles was also shown in a study. Their addition ($\approx 2.5\%$) into a CMC-chitosan-sodium alginate film reduced light transmittance in the 200-400 nm region, but not in the 400-600 nm region.⁵⁰ In a film of chitosan-CMC-oleic acid-Zn nanoparticles (0.5-2%), a significant reduction of transmittance in the 280 nm region and to a lesser extent of transmittance in the 600 nm region was noted. The authors suggested that the cause of this property of Zn nanoparticles is their quantum effect.³³

Antioxidant Properties

It was determined that the chitosan-nano TiO₂ (0.5%) packages had a value of 41.51% of the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical, compared to 27.13%, but using micro TiO₂. This demonstrates the importance of the nanometer scale.²¹

The antioxidant activity of a PLA film with the incorporation of Ag₂O, ZnFe₂O₄, Fe₂O₃ and WO₃ nanoparticles separately (0.5.% each) was also evaluated. The antioxidant capacity by the DPPH assay increased from 2.4 to 7% due to the transfer of free electrons from the nanoparticles to the free radicals in the nitrogen atom of the DPPH.³⁸ The same result was obtained in the evaluation of a nanocellulose-sodium alginate-CuO nanoparticles (1 and 5 mM) film but in this case, the electron transfer from the nanoparticles was to the nitrogen atom of the DPPH.⁷¹

The incorporation of TiO₂ nanoparticles (0.5%) into a sodium alginate-zein-betanine film did not increase its antioxidant activity.⁶¹ A peculiar case is the evaluation of a gelatin-TiO₂ nanoparticles (0.5-5% wt% of gelatin) film. Antioxidant activity increased (from ≈ 8 to $\approx 18\%$) when 0.5% of TiO₂ nanoparticles were used. At higher concentrations (1-5%), the antioxidant activity was reduced (up to $\approx 13\%$) by the adsorption of grape fruit seed extract on the nanoparticles, which prevented their interaction with ABTS and DPPH free radicals.⁶⁶

There is not much research on foods packaged with NBP-MNPs. For example, Cu nanoparticles were incorporated into hydroxy propyl methyl cellulose biopolymer for meat packaging, achieving a significant decrease in its microbial load during 15 days of storage at 4 °C.⁷² A CMS-chitosan-sodium alginate biofilm was fabricated with which red grapes were coated and stored for 7 days. The grapes shrank, showing an apparent significant weight (water) loss due to the elevated WVP values. This negative impact did not occur with the integration of ZnO nanoparticles into the film.⁵⁰ Shelf life of tomatoes coated with gelatin-CuO nanoparticles films and doped with TiO increased by more than 14 days.⁷³ Shelf life of red grapes was increased by 2 weeks when packed in a gelatin-chitosan-polyethylene glycol-Ag nanoparticles matrix.⁷⁴

Other results on increasing the shelf life of foods with NBP-MNPs include black grapes employing cellulose acetate phthalate-chitosan-ZnO nanoparticles film,⁷⁵ strawberries employing CMC-guar gum-Ag nanoparticles film,⁷⁶ mangoes employing PLA-bergamot essential oil-Ag and TiO₂ nanoparticles film,⁷⁷ guava employing chitosan-nettle leaf extract- ZnO and CuO nanoparticles film,⁷⁸ fresh blueberries employing chitosan-TiO₂ nanoparticles film,⁷⁹ cottage cheese employing PLA-TiO₂ and Ag nanoparticles film,⁸⁰ Egyptian white cheese employing CMC-chitosan-ZnO nanoparticles film,⁵⁵ and banana employing gum arabic-chitosan-ZnO nanoparticles film.²⁷

Despite not using NBPs, the incorporation of Cu nanoparticles into LDPE film increased the shelf life of an Indian dairy product.⁸¹ The LDPE film-ZnO nanoparticles maintained the quality of 'Hujingmilu' peaches during postharvest storage for 40 days at 2°C. This was achieved by alleviating chilling injury, maintaining fruit firmness and preventing browning due to inhibition of enzyme activity. Electrolyte loss was also prevented, and there was a decrease in O₂ and an increase in CO₂, among other beneficial effects.⁸² In an investigation, with the addition of TiO₂ in LDPE it was possible to maintain the quality of Chinese hickory during storage at 20°C, A high CO₂ and low O₂ environment was rapidly provided and the increase of peroxide and hexanal was delayed. With this, inhibition of peroxidase, lipoxygenase and lipase activities was achieved.²² Similarly, shelf life

of chicken breast fillets was increased by packaging them with a LDPE-Ag nanoparticles film,⁸³ shelf life of orange juice packaged with LDPE-Ag and ZnO nanoparticles was also increased,⁸⁴ and LDPE-ZnO nanoparticles film efficiently prolonged the shelf life of peaches.⁸²

In a comprehensive investigation, polyethylene-silver nanoparticles films was developed to package 432 samples of four different nuts, achieving shelf life of 18 months for walnuts, 18 months for hazelnuts, 19 months for almonds and 20 months for pistachios.⁸⁵

Current Status and Recommendations for Future Work

Despite the benefits offered by NBP-MNPs, the metal base remains under study due to its potential toxicity,⁸⁶ raising concerns and intrigue about its efficacy in the NBPs.⁸⁷ Migration of MNPs is more critical if the polymer is a coating because it has "greater contact" and accessibility to the cells of the food.

Many of the MNPs have been certified as GRAS by the FDA¹⁷ such as ZnO⁸⁸ and TiO₂.⁸⁹ On the other hand, some MNPs such as Ag can bioaccumulate in the testicles, liver, kidneys and brain.⁴⁸ However, according to the FDA, if the proportion meets the standards, they are safe for the consumer¹ and for the environment. In this sense, the EFSA establishes that the maximum release of Ag⁺ ion from package to food is ≥ 0.05 mg/kg.⁹⁰ The migration level of TiO₂ nanoparticles was evaluated in an α -chitosan film. Through incubation in food simulants for 10 days at 5 °C and 40 °C, it was determined that the amount of MNPs migrated to NBPs only in their ionic form was negligible ($< 5.44 \times 10^{-4}\%$).²¹ The migration of Ag nanoparticles in a polyvinyl chloride (PVC)-SiO₂ film. Olive oil, ethanol (50%) and acetic acid (3%) were used as food simulants and migration was determined to be < 30 , < 6 and 11 $\mu\text{g}/\text{kg}$, values well below the maximum limit established for metals in the Commission Regulation of the European Union.⁴³ A case of direct application of TiO₂ in doses exceeding the established limits (0.4-100 mg/mL) showed toxicological effects on different cell lines, proteins and animal models.⁹¹ It was also determined that the toxicity of CuO⁹² and Fe₂O₃⁹³ nanoparticles on human blood lymphocytes was concentration-dependent.

MNPs migration can also influence on the food quality and post-consumption in gastrointestinal microbiota due to their antimicrobial activity. An investigation had as results the inhibition of *Lactobacillus plantarum* by 82 and 95% with 50 $\mu\text{g}/\text{ml}$ of ZnO nanoparticles and Ag nanoparticles, respectively. In the case of *Lactobacillus fermentum* inhibition, Ag nanoparticles had the greatest effect, followed by ZnO nanoparticles.⁴ This is undesirable in foods produced by lactic fermentation such as yogurt, some vegetables and meats. Therefore, migration of NPMs during packaging could reduce the quality of these products, affecting their health benefits for the consumer. In a related study, active films based on LDPE with integration of Ag, CuO and Zn nanoparticles were developed for the packaging of Iranian white cheese. After 28 days of storage at 5 °C, there was a significant reduction of undesirable microorganisms such as *S. aureus*, total coliforms, molds and yeasts. However, the growth of lactic acid bacteria was also reduced, negatively influencing the physicochemical characteristics of the cheese.⁹⁴

Considering the above, an effective measure is needed to prevent migration and toxicity of MNPs.⁴⁶ The toxicity of MNPs can be reduced by making changes in their composition and structure.⁹⁵ Rutile-phase TiO₂ nanoparticles were found to be 100 times less cytotoxic than anatase-phase TiO₂ nanoparticles in different human cell lines.⁹⁶ Based on the literature, it is also advisable to apply encapsulation techniques, using materials such as cellulose⁴⁵ or zeolite.¹⁶ In a study, zeolite was used as an encapsulating material for ZnO and TiO₂ nanoparticles, managing to inhibit their migration to food.³⁸ Also, as an approach of the authors of this paper, it is desirable to use bimetallic or trimetallic bases to reduce the proportion of each nanoparticle. In addition, since each one would be used in lower concentration, migration can be reduced and even avoided. Similarly, more studies are needed on the migration of all MNPs with different approved doses, polymeric matrices and storage conditions. The potential negative effect of MNPs on the environment, food and consumer health should also be assessed to determine the safety and sustainability of their use. In addition, a safe way to assess the hazard of MNPs is through standards from international entities.⁹⁷

EFSA developed guidance related to the risk assessment of the use of nanomaterials in the various processes of the food industry.⁹⁸

Conclusions

According to multiple studies, the integration of MNPs such as ZnO, Ag and CuO into NBPs (from compounds such as chitosan, CMC and lignin) is performed in small proportions, generally 0.5% to 5%. MNPs significantly increase the antimicrobial activity of NBPs, in addition to improving their antioxidant, physical, mechanical, optical and barrier properties. This generates, in a sustainable way, an active and efficient packaging that improves food quality and prolongs shelf life. Although the amount of MNPs used is minimal, there are concerns about their migration and possible toxicological effects on consumer health and the environment. However, if the use of MNPs does not exceed the limits allowed

by the FDA, there is no risk, ensuring their safety in food packaging. Further studies on the use of MNPs at different doses are suggested to evaluate possible negative effects. The use of bimetallic and trimetallic bases is also recommended. In addition, although the technique is relatively new, its control should be prioritized so that, especially, the antimicrobial activity of MNPs does not affect the desirable bacteria and fungi of fermented foods such as yogurt during its packaging.

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Conflict of Interest

The author(s) declares no conflict of interest.

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