



Scientific Research

**Preparation biodegradable film of zinc oxide nanoparticles-carboxymethyl cellulose-gelatin and evaluation of its physical and mechanical properties**

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**ABSTRACT**

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In recent years, due to environmental risks associated with packaging material in the market, there has been an increasing tendency towards using natural and biodegradable polymers in product packaging. In this research, gelatin and carboxymethyl cellulose were used to produce Biodegradable packaging. In order to improve the mechanical properties and prevent destructive factors such as oxygen, carbon dioxide, and moisture, zinc oxide nanoparticles (ZnO) were used in the films (1, 3, and 5%), and the physical and mechanical properties of the produced films were evaluated. The results showed that using of different percentages of zinc oxide nanoparticles affected the mechanical and physical properties of the film. It was observed that with the increase in ZnO amount, the tensile strength of the samples increased and the elongation percentage decreased. Films without ZnO were flexible films and recorded the highest elongation values, and 5% ZnO samples had the lowest elongation percentage. The highest tensile strength was related to the sample containing 5% ZnO (35.93 MPa), and the lowest value was related to the sample without ZnO (29.3 MPa). It was also found that by increasing the amount of ZnO in the treatments, the permeability to water vapour, oxygen, and solubility decreased significantly, and the opacity of the films increased.

## 1- Introduction

Each year, a large amount of food is lost due to chemical spoilage, microbial growth, and reduced quality before it reaches consumers. It is clear that preventing and reducing waste during preparation, storage, processing, and distribution is essential. With advancements in food packaging industries, edible films have garnered more attention from researchers [1].

The materials used for food packaging are polymers derived from petroleum by-products. In recent years, due to the environmental risks posed by these packaging materials, there has been an increasing interest in using natural and biodegradable polymers as barriers against destructive factors such as oxygen, carbon dioxide, and moisture. Compared to synthetic polymers, these edible films and coatings not only provide the primary barrier properties of conventional packaging (resistance against gases, water vapor, and mechanical stress) but also, with changes in packaging conditions, can improve the shelf life, safety, and control of pathogens while preserving the food's quality [2].

Edible films are generally made from polysaccharides, lipids, and proteins. One important cellulose derivative is carboxymethyl cellulose, which is water-soluble and forms flexible and strong films. Gelatin, a protein source, is commonly used in the preparation of edible films. Gelatin's excellent film-forming capability, oxygen barrier properties, transparency, high flexibility, and strength are among its most notable features [3]. The excellent oxygen barrier of gelatin coatings makes them a suitable option for delaying lipid oxidation and mold growth [4].

The use of biopolymer films has faced limitations due to performance-related issues, inherent sensitivity to water, and relatively low strength and hardness, especially in humid environments. Previous studies have shown that films made from a single polymer are sensitive to environmental conditions and exhibit weaker mechanical properties [5]. Various methods exist for improving the barrier and mechanical properties of biodegradable films, one of which is combining different polymers to form composite films and mixing them with nanofillers.

Nanotechnology can address many challenges in the food industry. Nanocomposite polymers are an inexpensive, transparent, strong, heat-resistant, and oxygen-impermeable choice for packaging applications [6]. In this regard, the nanostructures

of specific metals like silver, gold, and zinc oxide have gained attention. To reduce costs in this field, the use of more affordable nanoparticles like zinc oxide (ZnO), which offers stable physical and chemical properties, antimicrobial characteristics, non-toxicity, and low cost, has become popular [7]. A study investigated the effect of zinc oxide nanoparticles (0.5%, 1.5%, and 3.5%) on improving the mechanical and thermal properties of gelatin-based films. Results showed that increasing the concentration of zinc oxide nanoparticles enhanced the tensile strength of the films and reduced elongation at break. The thermal stability of the films increased from 533°C (0.5% nanoparticles) to 559°C (1.5% nanoparticles) [8]. In another study, zinc oxide nanoparticles (1%, 3%, and 5% by weight) were added to polyvinyl alcohol. Results indicated that increasing the zinc oxide content to 3% by weight led to a 64% increase in tensile strength and a 72% increase in Young's modulus. The antibacterial properties of the layers improved with the addition of up to 5% zinc oxide by weight, though transparency decreased. The lowest water vapor permeability was observed in the sample containing 5% zinc oxide nanoparticles by weight [9]. In another study, the antimicrobial capacity of silver and zinc oxide nanoparticles (5% and 10% by weight) on certain quality characteristics of meat was examined. Meat was evaluated for storage periods of 0, 7, 10, 15, and 21 days. The composition of gases inside the packaging, some microbiological analyses, visual scores, and the lipid oxidation index (TBAR) were determined. With increasing storage time, the concentration of O<sub>2</sub> decreased, while the concentration and number of CO<sub>2</sub> and all types of microbes increased. It was observed that the addition of nanoparticles to the packaging significantly increased the antimicrobial effect of the film [7].

The aim of this study is to investigate the effect of adding zinc oxide nanoparticles to carboxymethyl cellulose-gelatin films and to evaluate the properties of the produced films.

## 2-Materials and Methods

**Materials:** The materials used included carboxymethyl cellulose, gelatin, glycerol, and zinc oxide nanoparticles, purchased from Faragostaran Sahand Company.

## 2.1 Film Preparation

To prepare a 1% (w/v) solution, 1 gram of carboxymethyl cellulose was added to 100 ml of distilled water and mixed using a magnetic stirrer at 600 rpm with a 4 cm cross-shaped magnet at 85°C for 45 minutes. To increase flexibility and soften the films, 40% glycerol based on the weight of the dry material was added to the solution and further mixed with the magnetic stirrer for 15 minutes to achieve uniformity. A 1.5% (w/v) gelatin solution was also prepared separately [4].

Based on previous studies, 1%, 3%, and 5% zinc oxide nanoparticles were dissolved in distilled water and subjected to ultrasound waves at 10 W for 20 minutes [9]. The nanoparticles were then added to the solution (50% carboxymethyl cellulose - 50% gelatin) and mixed for 45 minutes using a magnetic stirrer. The solution was transferred to Teflon containers and placed in an oven at 45°C for 20 hours to dry. A calculated amount of solution with a consistent volume of dry material was poured into the Teflon containers to ensure a uniform film thickness and eliminate the effect of thickness on the film properties.

## 2.2 Evaluation of the Mechanical Properties of the Films

For determining mechanical properties, rectangular samples (8×2 cm) were cut, and the tensile strength and elongation percentage at break were measured based on the ASTM method using a material testing machine (Hunsfield, England k-SH50) by analyzing the force-displacement curve [8]. The film thickness was measured using a micrometer.

## 2.3 Measurement of Film Solubility in Water

To measure the solubility of the films in water, pieces of the film were weighed and immersed in 50 ml of distilled water. They were stirred gently for 24 hours at 25°C, and the mixture was filtered through a filter paper. The remaining film on the filter was dried at 110°C until it reached a constant weight, and then it was weighed. The percentage of film solubility in water was calculated using

equation (1) [10].

$$\text{Solubility} = \frac{w_0 - w_f}{w_0} \times 100 \quad (1)$$

Where  $W_0$  is the initial weight before drying and  $W_f$  is the weight of the sample after immersion.

## 2.4 Measurement of Water Vapor Permeability (WVP)

The water vapor permeability (WVP) of the films was measured using a modified ASTM method [11]. Circular samples were placed over the lid of glass cups and placed in a desiccator containing silica gel. The water vapor transfer rate (WVTR) was determined from the increase in cup weight over time. A graph of weight gain vs. time was plotted, and the slope was calculated. The WVP was calculated from equation (2) [11].

$$\text{WVP} = \frac{\text{WVTR}}{P \times \Delta R \times X} \quad (2)$$

Where WVP is the water vapor permeability ( $\text{g/m s}^{-1} \text{Pa}^{-1}$ ), WVTR is the water vapor transfer rate,  $X$  is the film thickness,  $A$  is the internal surface area of the cup,  $P$  is the vapor pressure of pure water at 25°C, and  $\Delta R$  is the relative humidity difference between the cup and the desiccator.

## 2.5 Oxygen Permeability

Oxygen permeability of the films was measured using the ASTM method with an oxygen permeability testing device. The test was conducted at 25°C and 40% relative humidity. The device had a brass chamber to prevent gas leakage under a pressure of up to 6 bar. Circular samples (15×15 cm) with a thickness of 100  $\mu\text{m}$  were placed between the top and bottom of the brass chamber. Before each test, the bottom part of the chamber was evacuated, and the top part was filled with oxygen. The oxygen transfer rate (OTR) was measured by detecting the gas that passed through the film and increased the pressure in the lower part of the chamber [12].

## 2.6 Color and Transparency Measurement of

## Films

The surface color of the films was analyzed using a colorimeter (TES 135A Color Meter) to measure changes in the L (lightness), a (green-red spectrum), and b (blue-yellow spectrum) values of the films. After calibration, the color difference compared to the control sample (without nanoparticles) ( $\Delta E$ ) was calculated using equation (3) [13]. The opacity of the films was measured with a spectrophotometer at a wavelength of 600 nm, and opacity was calculated using equation (4) [14].

$$\Delta E = (\sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}) \quad (3)$$

$$\text{Opacity} = \frac{\text{value of absorbance at 600 nm/}}{\text{Film Thickness}} \quad (4)$$

## 2.7 Statistical Analysis

The software SPSS was used for comparing the factors and analyzing the significance between the prepared films using one-way ANOVA and Duncan's test.

## 3- Results and Discussion

The results of elongation percentage and tensile strength of the prepared films are shown in Table

Table 1. Mechanical properties of films with different ZnO contents

Film	Thickness ( $\mu\text{m}$ )	Elongation at break (%)	Tensile strength (Mpa)
0 ZnO	50.2 $\pm$ 7.62	14.2 $\pm$ 0.8 <sup>a</sup>	29.3 $\pm$ 1.5 <sup>a</sup>
1% ZnO	45.8 $\pm$ 7.35	12.1 $\pm$ 0.65 <sup>b</sup>	30.1 $\pm$ 1.09 <sup>a</sup>
3% ZnO	45.1 $\pm$ 4.18	11.2 $\pm$ 0.78 <sup>c</sup>	34.16 $\pm$ 1.16 <sup>b</sup>
5% ZnO	55.3 $\pm$ 4.18	6.9 $\pm$ 0.4 <sup>d</sup>	35.93 $\pm$ 1.08 <sup>b</sup>

In each column values assigned with different letters are significantly different ( $p < 0.05$ ).

In the food packaging industry, the color of produced films is a key factor affecting the product's appearance, consumer acceptance, and their application. In general, the more transparent

1. The results indicated that as the amount of ZnO increased, the tensile strength of the samples increased while the elongation percentage decreased. The films without ZnO were more flexible, recording the highest elongation percentage, while the 5% ZnO samples had the lowest elongation percentage. The highest tensile strength was found in the sample containing 5% ZnO (35.93 MPa), while the lowest was observed in the film without ZnO (29.3 MPa).

This result can be interpreted as the uniform distribution of zinc oxide nanoparticles in the film structure and the formation of ionic bonds between the nanoparticles and the biopolymer components, resulting in increased tensile strength. This strong ionic interaction between components reduces the mobility and elongation percentage of the film [15, 16]. It was also observed that increasing the nanoparticles from 3% to 5% did not significantly increase tensile strength, which may be due to excessive impurities in the film matrix at higher nanoparticle concentrations [17]. Packaged materials are exposed to environmental stresses, especially during transportation, highlighting the importance of increased tensile strength in packaging films. For final application, it is essential that the prepared film exhibits both high tensile strength and flexibility to withstand stresses and strains in various applications.

the polymer film is and the more it resembles synthetic polymer plastics, the better it is considered. The color indices of the films are shown in Table 2. As observed, the lightness (L) value of the sample without nanoparticles was

lower than that of the samples containing nanoparticles, which is consistent with the increased opacity resulting from the addition of nanoparticles to the film matrix. This result aligns with research on the effect of zinc oxide nanoparticles on the properties of gelatin-based biocomposite films [3]. The lightness values at all levels of zinc oxide did not differ significantly at the 5% level, although the highest lightness was observed in the 1% ZnO sample. The tendency towards yellowness (b) increased with higher levels of ZnO in the film matrix, with the highest value observed in the film containing 5% ZnO (3.2).

The total color difference, which indicates the color changes between nanoparticle-containing film samples and the control, is a measure used to assess the transparency changes of the films. Among the composite films, the color difference increased significantly with higher ZnO levels, likely due to the higher opacity of these films compared to the films without ZnO. The pure film (control) had lower opacity, and with the addition of nanoparticles, the transparency of the films decreased. This reduction in transparency may be due to the addition of nanoparticles to the film matrix, the aggregation of these nanoparticles, and the intrinsic property of zinc oxide nanoparticles to absorb light, making the films appear darker.[1]

Table 2. Colour indices of the films with different ZnO contents

Film	L	a	b	$\Delta E$	Transparency
0 ZnO	80.68±1.62 <sup>a</sup>	0.72±0.08 <sup>a</sup>	1.53± 0.72 <sup>a</sup>	10.58±1.75 <sup>a</sup>	0.62 <sup>a</sup>
1% ZnO	83.8± 0.62 <sup>b</sup>	0.02±0.08 <sup>b</sup>	1.48± 0.45 <sup>a</sup>	11.43±0.8 <sup>ab</sup>	0.93 <sup>b</sup>
3% ZnO	83.3± 0.38 <sup>b</sup>	0.06±0.04 <sup>b</sup>	2.1±0.35 <sup>b</sup>	12.23±0.42 <sup>b</sup>	0.96 <sup>b</sup>
5% ZnO	82.7±0.88 <sup>b</sup>	0.19± 0.09 <sup>b</sup>	3.2±0.56 <sup>c</sup>	14.25±1.15 <sup>c</sup>	1.17 <sup>c</sup>

In each column values assigned with different letters are significantly different ( $p < 0.05$ ).

Figure 1 shows the solubility and water vapor permeability of different treatments. It is clearly evident that as the amount of ZnO in the treatments increases, the solubility decreases significantly. However, the difference in solubility between the 3% and 5% nanoparticles was not significant. Solubility is one of the important physical parameters of biodegradable packaging films for preserving food during storage. The lower the solubility of the film, the better the film's properties. On the other hand, solubility can significantly impact the release rate of bioactive compounds. Most films based on polysaccharides and proteins have high solubility due to their hydrophilic nature [5]. High relative humidity in the environment or

storage area is one of the main factors contributing to food spoilage. It is clear that as the amount of ZnO in the treatments increases, water vapor permeability decreases significantly, which aligns with the results of other researchers [3, 6]. This can be attributed to the presence of nanoparticles uniformly dispersed in the polymer network, their crystalline nature, and the greater hydrophobicity of zinc oxide nanoparticles. Essentially, with the nanoparticles present, water vapor molecules must travel a longer and more complex path to pass through the film, which reduces the transfer and permeability rate of water vapor molecules. The more evenly and extensively the layers of nanoparticles are dispersed in the polymer matrix, the more these zigzag pathways increase, enhancing the film's barrier properties against water vapor and other gases [7].

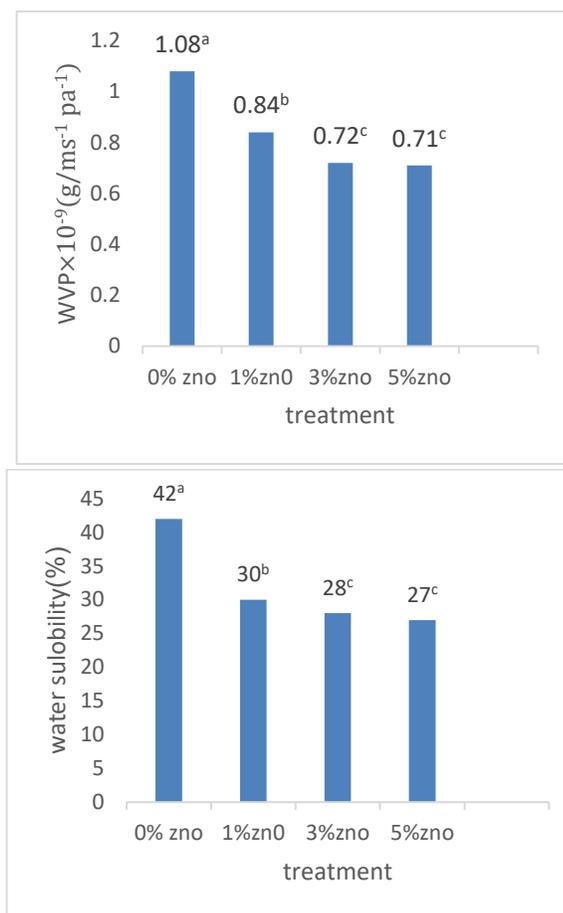


Fig. 1. Water solubility and water vapor permeability of the film samples with different ZnO contents

Due to the high density in the chains of biopolymer films, these films provide a good barrier against oxygen. Figure 3 shows the oxygen permeability in different treatments. It is observed that with the increase in nanoparticle concentration, the oxygen permeability decreases, which is consistent with the findings of Heidari and colleagues [8]. They demonstrated that oxygen permeability decreases as the concentration of titanium dioxide nanoparticles increases in composite films made from cassava starch and bovine gelatin.

Figure 4 shows the oxygen permeability in the 3% ZnO sample, where the permeability was measured

at  $0.87 \text{ cm}^3/\text{m}^2 \cdot \text{bar}$ . An oxygen permeability test is considered valid when there is no initial increase in pressure in the lower chamber of the oxygen permeability measurement device or when the increase is very minimal. This period is referred to as the "lag time." After this time, the rate of pressure increase over time becomes linear. If the pressure increase is linear from the very beginning, the results are not acceptable, indicating that the film has leaks [9].

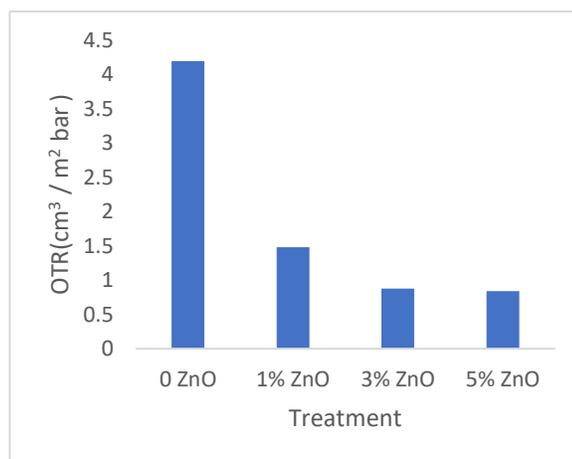


Fig. 3. Oxygen permeability of the film samples with different ZnO contents

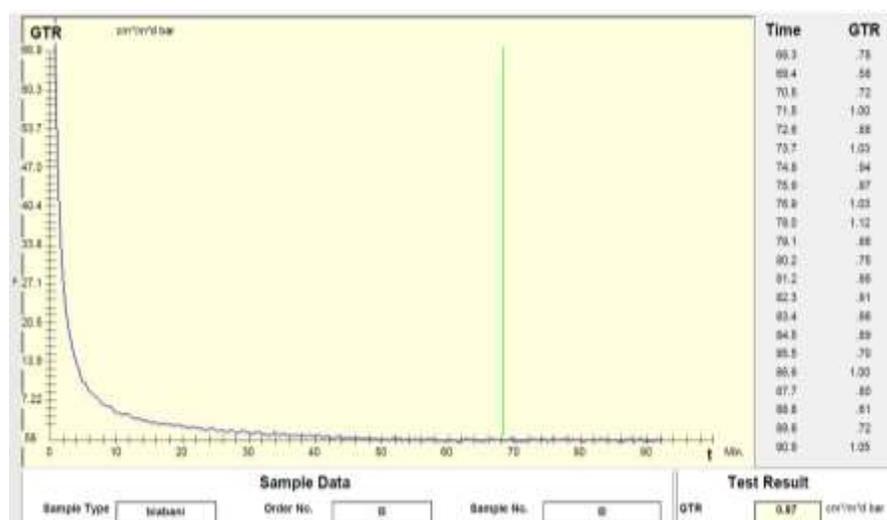


Fig. 4. Oxygen permeability of the 3% ZnO

#### 4-Conclusion

The biodegradable film made from zinc oxide nanoparticles, gelatin, and carboxymethyl cellulose has key unique features that make it a suitable alternative to petroleum-derived films for food preservation. The overall results of this study showed that using different percentages of zinc oxide nanoparticles affected the mechanical and physical properties of the film. The findings indicated that with an increase in ZnO content, the tensile strength of the samples increased while the elongation percentage decreased. It was also revealed that with the increase in ZnO content in the treatments, the solubility, and permeability to water vapor and oxygen significantly decreased.

#### Data Availability

The data used to support the finding of this study are available from the corresponding author upon request.

#### Conflict Of Interest

The authors have no conflicts interest to report.

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چکیده

اطلاعات مقاله

در سال های اخیر به دلیل مخاطرات زیست محیطی بسته های موجود در بازار، تمایل به استفاده از پلیمرهای طبیعی و زیست تخریب پذیر در نگهداری محصول بسته بندی شده بیشتر شده است. در این تحقیق از ژلاتین و کربوکسی متیل سلولز برای تولید بسته بندی زیست تخریب پذیر استفاده شد. برای تقویت ویژگی های مکانیکی و ممانعت در برابر عوامل مخرب از قبیل گاز اکسیژن، دی اکسید کربن و رطوبت، به فیلم ها نانو ذرات اکسید روی (ZnO) در سطوح (۱، ۳ و ۵ درصد) اضافه گردید و در نهایت خصوصیات فیزیکی و مکانیکی فیلم های تولیدی مورد ارزیابی قرار گرفت. نتایج نشان داد که استفاده از درصد های متفاوت نانو ذرات اکسید روی بر خصوصیات مکانیکی و فیزیکی فیلم تأثیر گذاشت. همچنین مشاهده شد با افزایش مقدار ZnO مقاومت کششی نمونه ها افزایش و درصد افزایش طول کاهش یافت. فیلم های بدون ZnO فیلم های منعطفی بوده و بیشترین مقادیر افزایش طول را ثبت نمودند و نمونه های ZnO 5% کمترین درصد افزایش طول را داشتند. بالاترین استحکام کششی مربوط به نمونه حاوی ZnO 5% (۳۵/۹۳ Mpa) و پایین ترین مقدار مربوط به نمونه بدون ZnO (۲۹/۳ Mpa) بود. هم چنین مشخص شد که با افزایش میزان ZnO در تیمارها میزان نفوذپذیری به بخار آب، اکسیژن و حلالیت به طور معناداری کاهش و میزان کدورت فیلم ها افزایش پیدا کرد

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