Development of Antibacterial Carboxymethyl Cellulose-Based Nanobiocomposite Films Containing Various Metallic Nanoparticles for Food Packaging Applications

Yadollah Ebrahimi, Seyed Jamaleddin Peighambardoust, Seyed Hadi Peighambardoust, and Sirous Zahed Karkaj

Abstract: In this study, silver (Ag), zinc oxide (ZnO), and copper oxide (CuO) metallic nanoparticles were used in preparation of carboxymethyl cellulose (CMC) nanobiocomposite films. Scanning electron microscopy (SEM), X-ray diffraction (EDXA), water vapor permeability (WVP), ultraviolet and visible light (UV–Vis) spectroscopy, and mechanical and microbial tests were used to determine the characteristics of the obtained active films. SEM results showed that the CMC nanobiocomposite films had roughness deflection levels and the EDXA test confirmed the presence of Ag, ZnO, and Cuo nanoparticles in the biopolymer tissue. UV–Vis spectroscopy confirmed that with addition of metallic nanoparticles to the pure CMC film, absorption rate increased and WVP decreased. In the mechanical tests, addition of nanoparticles also increased the tensile strength of the films, and the nanobiocomposite films exhibited higher resistance compared to the pure CMC film. Films incorporating metallic nanoparticles showed antibacterial properties against *Escherichia coli* and *Staphylococcus aureus* growth. Thus, nanobiocomposite films can be used as active packaging films and could increase the shelf-life of the food.

Keywords: Ag, ZnO, and CuO nanoparticles, antibacterial properties, carboxymethyl cellulose, food packaging, nanobiocomposite film

Introduction

Active packaging is classified as a subset of intelligent packaging that increases the life of food in the presence of special add-ons in packaging or in-pack films. The development of active packaging has led to many improvements in cases such as delaying oxidation and controlling respiration rates, bacterial growth, moisture migration, carbon dioxide absorption and emitter, odor absorbers, ethylene eliminators, and odoriferous substances (Hu, Wang, & Wang, 2016; Youssef, El-Sayed, El-Sayed, Salama, & Dufresne, 2016). The use of biopolymers such as carboxymethyl cellulose (CMC) improves some of the problems of synthetic plastics such as environmental pollution, but because of the hydrophilic properties of polysaccharides and proteins, the use of these materials in their applications is very limited (Han & Wang, 2017; Rakhshaei & Namazi, 2017). Developments have been made with the introduction of nanotechnology in the packaging industry, which opened the route for industrial manufacturers and researchers to overcome many of the problems and limitations of synthetic polymers and biopolymers. Nanoparticles used in food packaging are classified into organic and inorganic; nanoparticles such as silver, titanium, zinc oxide (ZnO), copper oxide (CuO), iron, clay, and silica are part of inorganic nanoparticles. Films made of nanomaterials and biopolymers, or so-called nanobiocomposites, exhibit more favorable properties. Improvement of mechanical properties and enhancing antimicrobial properties are among these characteristics (Baltić et al., 2013; Emamifar, Kadivar, Shahedi, & Soleimanian-Zad, 2010; Fasihnia, Peighambardoust, Peighambardoust, & Oromiehie, 2018; Oun & Rhim, 2017). The migration of nanoparticles is an important topic in their application in human and environmental toxicology. In nanobiocomposites containing nanoparticles, because the amount of nanoparticles used is lower and is below 5% wt., even if their migration have been occurred, this migration cannot affect the safety of the materials in contact with them. Among the metal cations, silver ions are known to possess the most antimicrobial properties against a wide range of microorganisms in addition to microbes; it also affects fungi and viruses. Silver nanoparticles can damage microorganisms by forming a cavity on the surface of the cell membrane. In addition, they may penetrate the cell to damage the DNA (Brody, Strupinsky, & Kline, 2001; Khodaeimehr, Peighambardoust, & Peighambardoust, 2018; Llorens, Lloret, Picouet, Trbojevich, & Fernandez, 2012). In the case of copper nanoparticles, it can be said that the important factor in the formation of antimicrobial properties of copper is its ability to capture of electrons, so that it is highly capable to catalyze oxidation and reduction reactions. When copper is in the oxide state, due to interference with nucleic acids, the active site of enzymes and cell wall components causes the death of microbial cells (Bruna, Peñaloza, Guarda, Rodríguez, & Galotto, 2012; Cioffi et al., 2005; Peighambardoust, Beigmohammadi, & Peighambardoust, 2016). Zinc nanoparticles also have antimicrobial properties and have advantages such as low cost, white appearance, and resistance to UV radiation as compared to silver. ZnO is used for its high antimicrobial properties against a wide range of microorganisms in everyday applications such as medicines, cosmetics, and medical equipment (Li, Xing, Jiang, Ding, & Li, 2009). Many studies have been done on the simultaneous use of nanoparticles in food packaging, which can be attributed to the synergistic effect of these nanoparticles (Fasihnia,

JFDS-2019-0553 Submitted 4/14/2019, Accepted 7/4/2019. *Authors Ebrahimi and Peighambardoust are with Faculty of Chemical and Petroleum Engineering, Univ. of Tabriz, Tabriz, Iran. Author S. H. Peighambardoust is with Dept. of Food Science, College of Agriculture, Univ. of Tabriz, Tabriz, Iran. Author Karkaj is with Faculty of Engineering, Islamic Azad Univ., Ahar Branch, Ahar, Iran. Direct inquiries to author S. J. Peighambardoust (E-mail: j.peighambardoust@tabrizu.ac.ir).*

doi: 10.1111/1750-3841.14744

Journal of Food Science A Publication of the Institute of Food Technologists

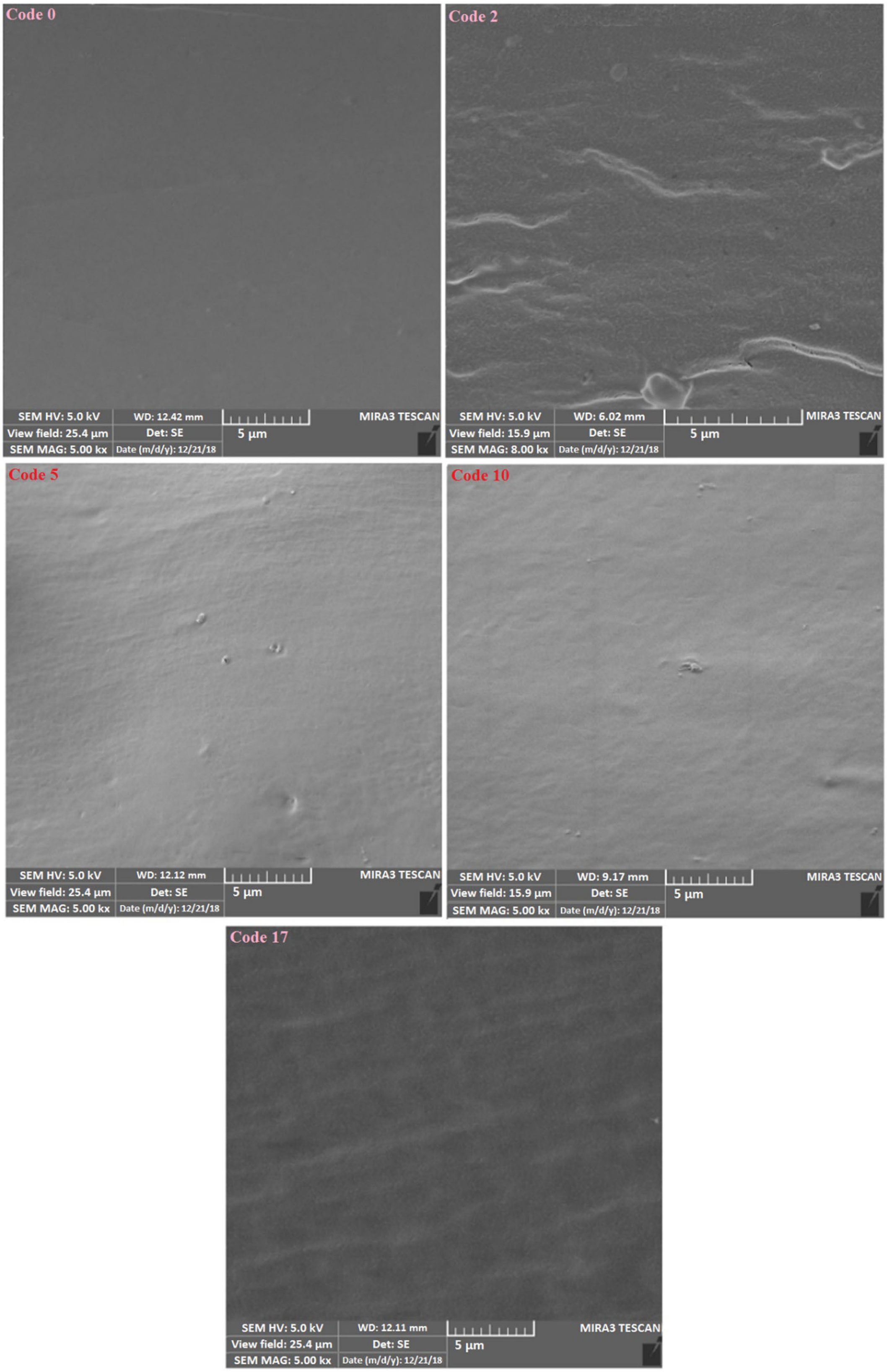
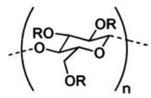


Figure 1–Images of scanning electron microscopy of pure CMC film (code 0), CMC films containing ZnO, CuO, and Ag, each equal to 2% wt. (codes 2, 5, and 10, respectively), as well as CMC film containing a mixture of ZnO, CuO, and Ag, each equal to 0.667% by weight (code 17).

Table 1–Materials required for the preparation of CMC nanocomposites containing metal nanoparticles.

Materials	Chemical formula	Molecular weight (g/mol)	Density (g/cm^3)	Specific surface (m^2/g)	Nanoparticle size (nm)	Supplier company
Carboxymethyl cellulose		41,000	1.6	–	–	Nippon Japan, Tokyo, Japan
Zinc oxide	ZnO	81.41	5.61	30–35	20–30	Neutrino Co., Tehran, Iran
Copper oxide	CuO	79.55	6.51	80	35	Neutrino Co., Tehran, Iran
Silver	Ag	107.81	10.49	10.4	<50	Neutrino Co., Tehran, Iran
Glycerol	$C_3H_8O_3$	92.09	1.26	–	–	Merck Chemicals Co., Darmstadt, Germany

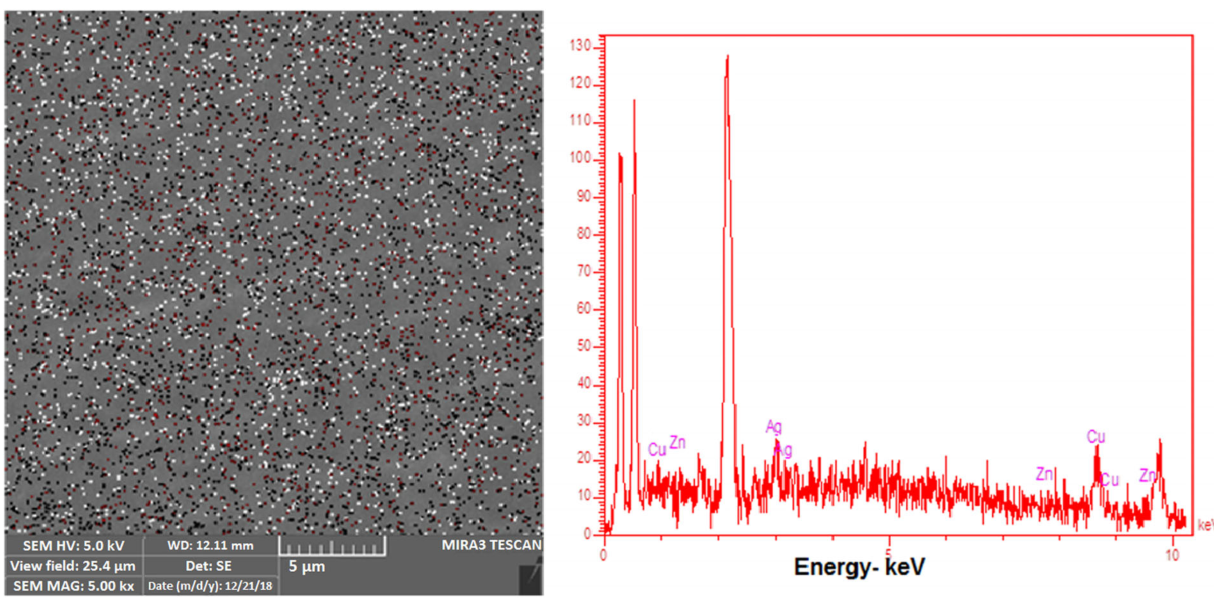


Figure 2–The results of the X-ray energy emission analysis along with the X-ray map for a CMC nanobiocomposite film containing a mixture of ZnO, CuO, and Ag nanoparticles, each equal to 0.667 wt. (code 17).

Peighambardoust, & Peighambardoust, 2018). However, in some cases, they can have negative effects on each other. Combined use of Ag nanoparticles and nanoparticles of ZnO and CuO as an antimicrobial compound in the formulation of some coatings has also been carried out to evaluate the antimicrobial property of these compounds on different strains of microorganisms (Muppalla, Kanatt, Chawla, & Sharma, 2014; Wang, Zhang, Zhang, & Li, 2012; Yadollahi, Gholamali, Namazi, & Aghazadeh, 2015; Yoksan & Chirachanchai, 2010). Nevertheless, no study has been conducted on the simultaneous use of these compounds in biopolymers. In this study, for the first time we investigated various properties of nanobiocomposite films based on CMC containing nanoparticles of Ag, ZnO, and CuO and films containing a combination of these nanoparticles.

Experiments

Materials

Table 1 shows all materials used in the preparation of CMC nanobiocomposites containing metal nanoparticles in this study. All materials used had a purity of over 99% and were used without any purification. For microbiological analysis, two types of pathogenic bacteria, gram-positive *Staphylococcus aureus* ATCC-14 458 and gram-negative *Escherichia coli* O157:H7 ATCC-11775, obtained from the collection center of Industrial Microorganisms (Tehran, Iran) were used. To cultivate these two microorganisms, specific culture media Wyolt Red Beel Dextrose Agar were used for *E. coli* and Manitol Salt Agar medium for *S. aureus*. Manitol salt agar has been obtained from Mirmadia (Tehran, Iran) and the Wyolt Red Beel Agar culture media from Charlero (Milan, Italy). In microbiological analyzes, sodium chloride salt of Mirmidia Corporation was used to prepare the serum of physiology in the preparation of dilutions. Charlero's broth nutrient medium was also used to propagate microorganisms to reach its original value.

Method for the preparation of CMC-based nanobiocomposites containing metal nanoparticles

In the preparation of CMC-based nanobiocomposite films containing metallic nanoparticles, solution casting method was used, which consists of three steps of dissolving the biopolymer in the

Table 2–Type of nanoparticles and the percentage composition used in the preparation of CMC-based nanobiocomposites.

Film sample code	Nanobiocomposite film	Metal nanoparticle type	Weight of metal nanoparticles (% wt.)
0	Pure CMC (As a blank sample)	–	0
1	CMC: ZnO	ZnO	1
2	CMC: ZnO	ZnO	2
3	CMC: ZnO	ZnO	3
4	CMC: CuO	CuO	1
5	CMC: CuO	CuO	2
6	CMC: CuO	CuO	3
7	CMC: Ag	Ag	0.5
8	CMC: Ag	Ag	1
9	CMC: Ag	Ag	1.5
10	CMC: Ag	Ag	2
11	CMC: (Ag + ZnO)	Ag + ZnO	1 + 1
12	CMC: (CuO + ZnO)	CuO + ZnO	1 + 1
13	CMC: (Ag + CuO)	Ag + CuO	1 + 1
14	CMC: (Ag + ZnO)	Ag + ZnO	0.667 + 1.333
15	CMC: (CuO + ZnO)	CuO + ZnO	1.332 + 0.667
16	CMC: (CuO + ZnO)	CuO + ZnO	0.667 + 1.332
17	CMC: (Ag + ZnO + CuO)	Ag + ZnO + CuO	0.667 + 0.667 + 0.667
18	CMC: (Ag + ZnO + CuO)	Ag + ZnO + CuO	1.333 + 0.333 + 0.333
19	CMC: Ag	Ag	3

Table 3–Diameter of the inhibition zone of pure CMC and nanobiocomposite films containing Ag, ZnO, and CuO nanoparticles in the microbial test against *S. aureus* and *E. coli*.

Nanobiocomposite film / Diameter of the inhibition zone	Film sample code	Weight of metal nanoparticles (% wt.)	*Escherichia coli* (mm)	*Staphylococcus aureus* (mm)
Pure CMC (As a blank sample)	0	0	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$
CMC - Ag	7	0.5	$0.0^{c} \pm 0.0$	$0.0477^{b} \pm 0.2868$
CMC - Ag	8	1	$0.0503^{bc} \pm 0.2233$	$0.0225^{cd} \pm 0.4608$
CMC - Ag	9	1.5	$0.0159^{b} \pm 0.2833$	$0.0520^{bc} \pm 0.5575$
CMC - Ag	10	2	$0.1761^{b} \pm 0.5833$	$0.0574^{b} \pm 0.6842$
CMC - Ag	19	3	$0.0829^{a} \pm 0.6875$	$0.1384^{a} \pm 1.1317$
CMC - ZnO	1	1	$0.0375^{b} \pm 0.1783$	$0.0138^{c} \pm 04817$
CMC - ZnO	2	2	$0.0213^{b} \pm 0.2183$	$0.0188^{b} \pm 0.5492$
CMC - ZnO	3	3	$0.0375^{a} \pm 0.2975$	$0.0188a \pm 0.6192$
CMC - CuO	4	1	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$
CMC - CuO	5	2	$0.0144^{b} \pm 0.183$	$0.0^{c} \pm 0.0$
CMC - CuO	6	3	$0.0120^{a} \pm 0.2300$	$0.0^{c} \pm 0.0$

Note. Data are mean of triplicate measurements. Different letters in each column indicate significant ($P < 0.05$) differences between means in Tukey's test.

appropriate solvent, the casting of the resulting polymer solution, and drying. Noteworthy is the uniform dispersion of nanoparticles in the biopolymer films, and prevent their aggregation. To prevent nanoparticle aggregation and uniform distribution in the CMC matrix, different weight percentages of nanoparticles (Ag, ZnO, and CuO—1%, 2%, and 3% wt.%, respectively) compared to dry CMC in 150 mL distilled water were spread and placed in an ultrasonic bath for 3 to 4 hr. Then, 5 g of CMC and glycerol (40 % w/w dry CMC) were added to the same 100 mL of solution. The solution was then heated to 75 °C for 30 min in an oil bath with stirring and then CMC gelatin was prepared. In order to leave any air bubbles from the solution, the mixture was cooled to 40 °C and stirred slowly for 60 min, and then 25 mL of the solution was poured into the Petri dish polystyrene plate and placed in an oven at 35 °C for 15 hr to make a final nanobiocomposite films.

Characterization of nanobiocomposite films

Scanning electron microscopy analysis with energy-dispersive X-ray spectroscopy. Microstructure of CMC and nanobiocomposite films was characterized using scanning electron microscopy (SEM). Morphology of surface of CMC and nanobiocomposite films was observed using an SEM (1430VP L, Germany) operated at 5 kV. The surfaces were coated with a thin layer of gold prior to microscopic imaging. The energy-dispersive X-ray spectroscopy (EDXA; X-ray dot mapping) has also been used to study the distribution of metal nanoparticles in a polymer matrix.

Mechanical strength of nanobiocomposite films. The rectangular shaped specimens of 2 cm wide and 6 cm long were cut from films and used for the tensile tests. The elastic modulus, tensile strength, and elongation at break of the films were determined using Zwick/Roell FR010 tensile testing machine according to the ASTM-D882 standard method. The tensile test was performed at room temperature with crosshead speed of 5 mm/min. At least three specimens were tested for each composition and the average values were reported. A hand-held micrometer apparatus (No. 7326, Mitutoyo Manufacturing Co., Ltd., Tokyo, Japan) was used to measure the film thickness. An average value of thickness obtained from five measurements made on each specimen was used in tensile properties and water vapor permeability (WVP) calculations. All film samples were preconditioned for 48 hr at 25 °C and Relative humidity (RH) = 50% before testing. Three replications were used to determine each film property.

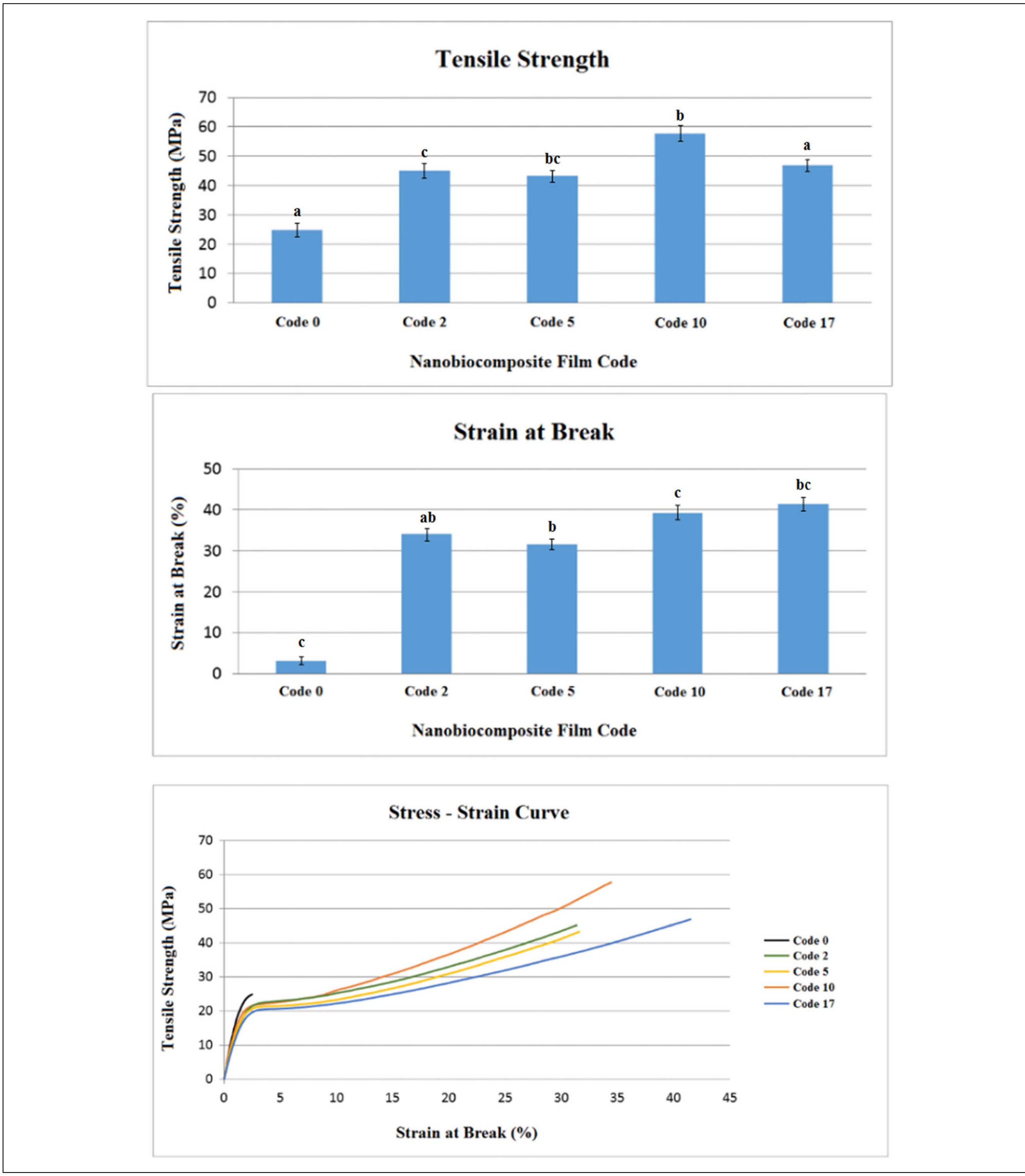


Figure 3–Ultimate strength and strain strength to the failure of pure CMC film (code 0), CMC films containing ZnO, CuO, and Ag equal to 2% wt. (codes 2, 5, and 10, respectively), and CMC film containing a mixture of ZnO, CuO, and Ag nanoparticles each equal to 0.667% wt. (code 17). Different letters in each figure represent significant ($P < 0.05$) differences between means using Tukey's test.

Measurement of UV absorption of nanobiocomposite films. The transparency of the films was measured by the Japanese UV-1700 Shimadzu UV–Vis spectrophotometer. In this study, the transparency of films was measured according to ASTM D1746 standard. The films were cut into 3 × 2 cm pieces and placed for a relative humidity of 55% at 25 °C for 24 hr. Then, on one of the two radial spectrophotometers, sample was placed, and in the other tube, the air is placed as a reference. The amount of light absorption in the wavelength range of 200 to 800 nm was measured and its passage rate was compared with pure CMC and the data were plotted on the computer using Excel software.

WVP of nanobiocomposite films. To measure the WVP of pure CMC film and CMC nanobiocomposite films containing metal nanoparticles, the ASTM E96 method was used. For this

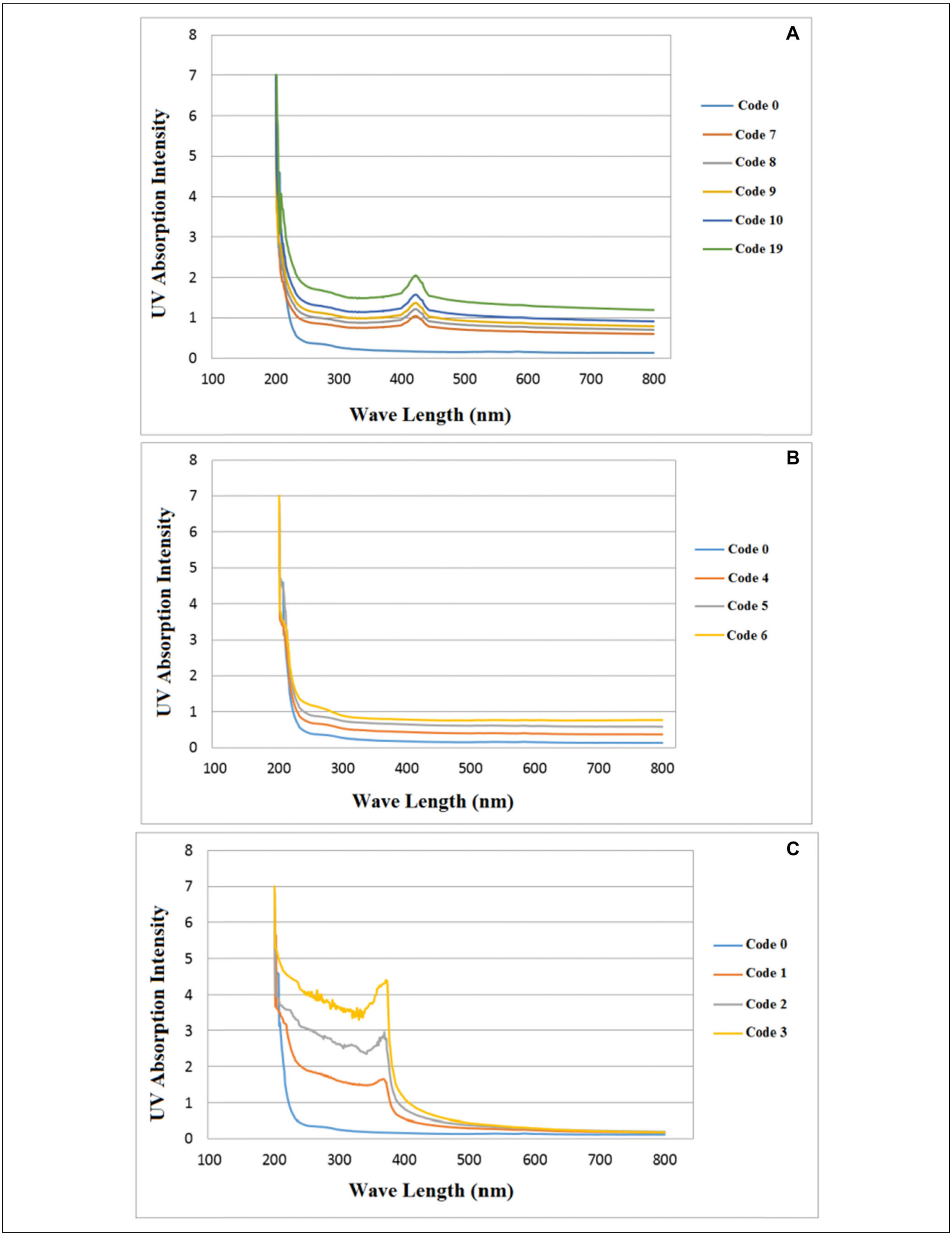


Figure 4–Ultraviolet absorption graph of CMC nanobiocomposite films containing single nanoparticles (A) Ag, (B) CuO, and (C) ZnO at various levels of weight.

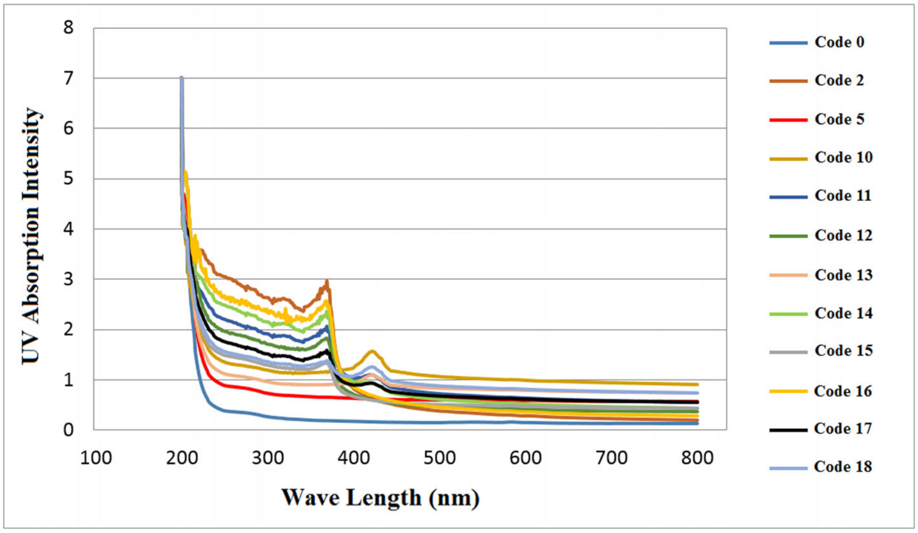


Figure 5–Ultraviolet absorption of CMC films containing a mixture of Ag, ZnO, and CuO nanoparticles at 2% wt.

Table 4–Diameter of the inhibition zone of pure CMC and nanobiocomposite films containing a mixture of Ag, ZnO, and CuO nanoparticles at 2% wt. in the microbial test against *S. aureus* and *E. coli*.

Diameter of the inhibition zone / Nanobiocomposite film	Film sample code	Weight of metal nanoparticles (% wt.)	*Escherichia coli* (mm)	*Staphylococcus aureus* (mm)
Pure CMC (As a blank sample)	0	0	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
CMC - ZnO	2	2	0.0213 ± 0.2183	$0.0188^{b} \pm 0.5492$
CMC - CuO	5	2	$0.0144^{b} \pm 0.1983$	$0.0^{c} \pm 0.0$
CMC - Ag	10	2	$0.1761^{a} \pm 0.5833$	$0.0574^{b} \pm 0.6842$
CMC - (Ag + ZnO)	11	1 + 1	$0.0076^{def} \pm 0.3783$	$0.1236^{b} \pm 0.5108$
CMC - (CuO + ZnO)	12	1 + 1	$0.0096^{bcde} \pm 0.1792$	$0.1277^{ab} \pm 0.3167$
CMC - (Ag + CuO)	13	1 + 1	$0.005^{ef} \pm 0.3500$	$0.0559^{c} \pm 0.4675$
CMC - (Ag + ZnO)	14	0.667 + 1.333	$0.0087^{bc} \pm 0.3818$	$0.0559^{a} \pm 0.6767$
CMC - (CuO + ZnO)	15	1.333 + 0.667	$0.006^{cdef} \pm 0.1238$	$0.0437^{cd} \pm 0.1733$
CMC - (CuO + ZnO)	16	0.667 + 1.333	$0.0536^{bc} \pm 0.2642$	$0.0677^{cd} \pm 0.1308$
CMC - (Ag + ZnO + CuO)	17	0.667 + 0.667 + 0.667	$0.0075^{b} \pm 0.3590$	$0.0881^{a} \pm 0.7392$
CMC - (Ag + ZnO + CuO)	18	0.333 + 0.333 + 1.333	$0.0275^{bc} + 0.5767$	$0.0763^{ab} + 0.6933$

Note. Data are mean of triplicate measurements. Different letters in each column indicate significant ($P < 0.05$) differences in Tukey's test.

purpose, vials with a diameter of 1.5 cm and a height of 4.5 cm were used. An amount of 3 g of calcium sulfate was placed in vials and a piece of the film was placed on the vial. The vials are weighted with all of their contents and placed in a desiccator containing potassium sulfate saturated solution. To ensure preservation of the saturation state, it is allowed to create some sedimentation of potassium sulfate in the bottom of the desiccator. Saturated potassium sulfate at 25 °C produces a relative humidity of 97%. The desiccator was placed in a 25 °C incubator and the vials weight was measured for 7 days, every few hours. The amount of water vapor transferred from the films is determined by the weight gain of the vials. The weight gain curves were plotted over time and after linear regression, the slope of the line was calculated. The steam transfer rate was obtained from the slope division of each vial to the film surface exposed to water vapor transmission as shown by Equation (1). As shown by Equation (2), the division of water vapor transfer rate (WVTR) to the vapor pressure difference on both sides of the film resulted in the permeability of film samples compared to WVP. Due to the presence of potassium sulfate in the vial, vapor pressure inside the vial is considered zero. The vapor pressure of the outside film was also obtained from the RH in the desiccator (97%) and pure water vapor pressure at 25 °C. The product of the WVTR and the thickness of the film also obtained the film permeability to water vapor. This test was repeated three times for each specimen (Yoksan & Chirachanchai, 2010).

$$j = \mathrm{WVRT} = \frac{\Delta W}{T \times A} \tag{1}$$

Here, j (WVTR) is the water vapor flux in the film, also called the water vapor transfer rate (g/m^2·hr); ΔW is the amount of water vapor passed through the film (g); T is the duration of water vapor transmission (hr); and A is the film surface area (m^2).

$$\mathrm{WVP} = \frac{\mathrm{WVRT} \times X}{P\,(R_2 - R_1)} \tag{2}$$

Here, P is equal to pure water vapor pressure at 25 °C (3,169 Pa), R_2 is equal to RH inside the desiccator (97%), and R_1 is equal to RH inside the vial (0%).

Antibacterial properties of nanobiocomposite films. Microbial tests were carried out on *E. coli* and *S. aureus*, which are representative of gram-negative and gram-positive bacteria, respectively. First, the microorganisms were cultured in a sterile Tryptic Soy Broth culture medium (30 g/L distilled water) and incubated at 37 °C for 24 hr to reach microorganisms to the

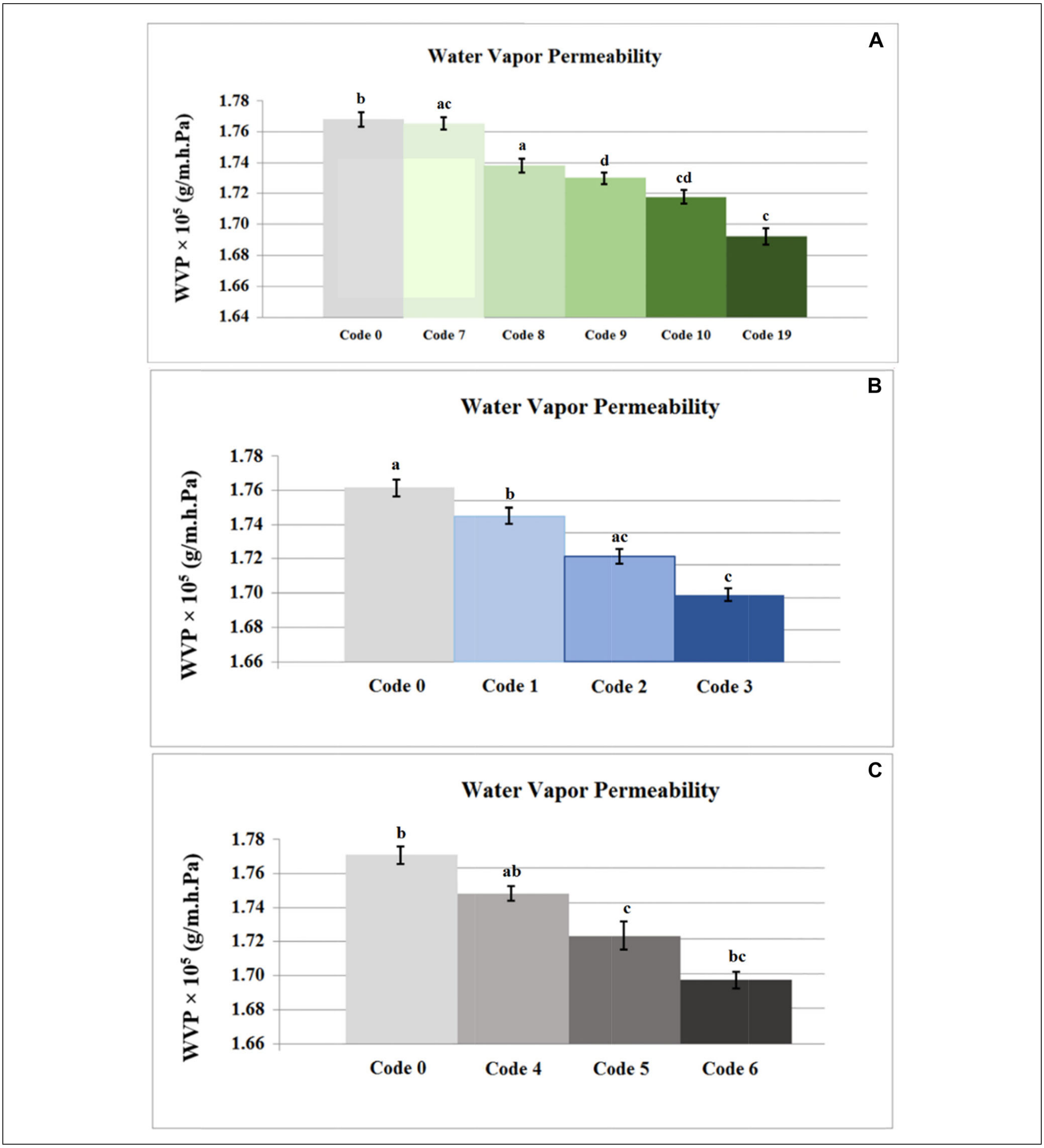


Figure 6–Water vapor permeability of pure CMC film and CMC films containing (A) Ag, (B) ZnO, and (C) CuO at various weight concentration. Data are mean of triplicate measurements. Error bars indicate standard deviations. Different letters in each figure represent significant ($P < 0.05$) differences between means using Tukey's test.

desired initial numbers (initial bacterial count *E. coli* was 1.76×10^9 CFU/mL and the primary number of *S. aureus* was 5.8×10^8 CFU/mL). The agar diffusion method (inhibition zone test) was used to investigate the antibacterial properties of CMC-based biopolymer films. In this diffusion, the antibacterial properties of nanobiocomposite films containing metal nanoparticles were studied. In the case of release of an antibacterial agent, the film contained in the agar medium containing target microorganisms, an area of inhibition is formed around it; the presence of this region indicates the release of antibacterial material from the film, resulting in the lack of growth of microorganisms in the area. The larger the containment area, the more antibacterial material is released from the film. In order to test the inhibitory range, appropriate culture media for each microbe need to be used for *S. aureus* from MSA medium (111 g per liter of distilled water) and for *E. coli* from VRBD medium (39.5 g per liter distilled water) were

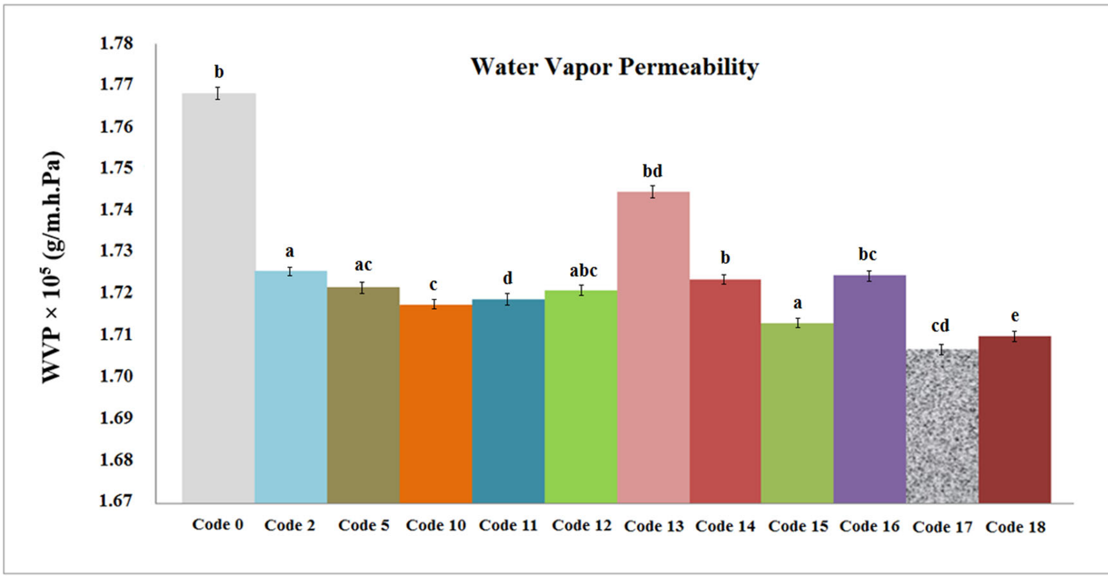


Figure 7–Water vapor permeability for pure CMC film and CMC films containing a mixture of Ag, ZnO, and CuO nanoparticles at 2% wt. Data are mean of triplicate measurements. Error bars indicate standard deviations. Different letters in each figure represent significant ($P < 0.05$) differences between means using Tukey's test.

used. After preparing the culture media in 8 cm diameter plates, the microorganism was poured onto the medium at 0.1 mL, the sterilized L-shaped rod was spread over the medium, and the films prepared in 2 cm were placed on medium and then moved inside the incubator. The inhibitory range was measured after 24 hr.

Experimental design and statistical analysis. In this study, Minitab 16 experimental design software was used to analyze the data and optimize the composition of nanoparticles to create an optimal antibacterial property. For statistical analysis of data from microbial analysis of nanobiocomposite films, one-way ANOVA analysis and evaluation software was used at 5% probability ($P < 0.05$). Tukey's test was used to confirm the existence of a significant difference between the means. The most important issue of this research is to study the main and mutual effects of nanoparticles in their combined use, which is why the experimental design software has been used to determine the optimal number of experiments. All treatments were performed in microbiological analyzes in three replicates. Table 2 represents a list of percent composition, and type of nanoparticles obtained from the design of the experiments is used in the preparation of antimicrobial nanobiocomposites in this study.

Results and Discussion

SEM analysis with EDXA

Study of the surface morphology of CMC and nanobiocomposite films prepared from the SEM are used and the microstructure images were shown in Figure 1. These images relate to the level of breakdown of pure CMC film (control) and its nanobiocomposite films. Figure 1A shows a very smooth and homogeneous surface morphology at the level of pure CMC film failure, which is a sign of the highly ordered morphology of its biopolymer matrix. Morphology of nanobiocomposite films containing nanoparticles is even sharper and heterogeneous than control, and this may be due to the effect of the mass of some nanoparticles in the preparation of nanobiocomposites (Espitia et al., 2013). Furthermore, by comparing the images in Figure 1B, 1C, and 1D, it is clear that the nanobiocomposite film surface containing ZnO and CuO is sharper and more heterogeneous than those of Ag films, which indicates better diffusion and widespread silver nanoparticles in CMC biopolymer, and the effects of nanoparticle massification in ZnO and CuO have been more severe, which is consistent with previous studies (Yoksan & Chirachanchai, 2010). Figure 2 shows the results of the X-ray energy emission analysis along with the X-ray map for a CMC nanobiocomposite containing a combination of ZnO, CuO, and Ag nanoparticles, each equal to 0.667% wt.% (code 17). The white, red, and black points indicate the diffusion of ZnO, CuO, and Ag nanoparticles, respectively. The distribution of color points in the dot-mapping image shows a uniform and nonmassive playback of the biopolymer matrix.

Mechanical strength of nanobiocomposite films

Purpose of adding nanoparticles in biopolymer films is to improve certain properties, including mechanical properties to use in food packaging. Ultimate tensile strength and strain to failure of nanobiocomposite films containing three types of ZnO, CuO, and Ag nanoparticles at 2% wt. at ambient temperature were measured and the results are shown in Figure 3. With the addition of single and combined nanoparticles of ZnO, CuO, and Ag to 2% wt. into the biopolymer matrix, nanobiocomposite films containing metal nanoparticles exhibit better mechanical properties than pure CMC films in packaging applications. Depending on the level of interfacial interactions between the filler nanoparticles and the matrix, how the nanoparticles are dispersed inside the matrix, the amount of empty spaces, and differences in tensile strength are observed for nanobiocomposite films. The tensile strength and strain rate up to the breaking point of the film containing silver nanoparticles are much better than other nanobiocomposite films. This is probably due to the strong interactions between the CMC chains and silver nanoparticles, as well as the filling of empty spaces between the chains. SEM images also confirm this interaction in the smooth morphology of the surface of nanobiocomposite films containing silver nanoparticles.

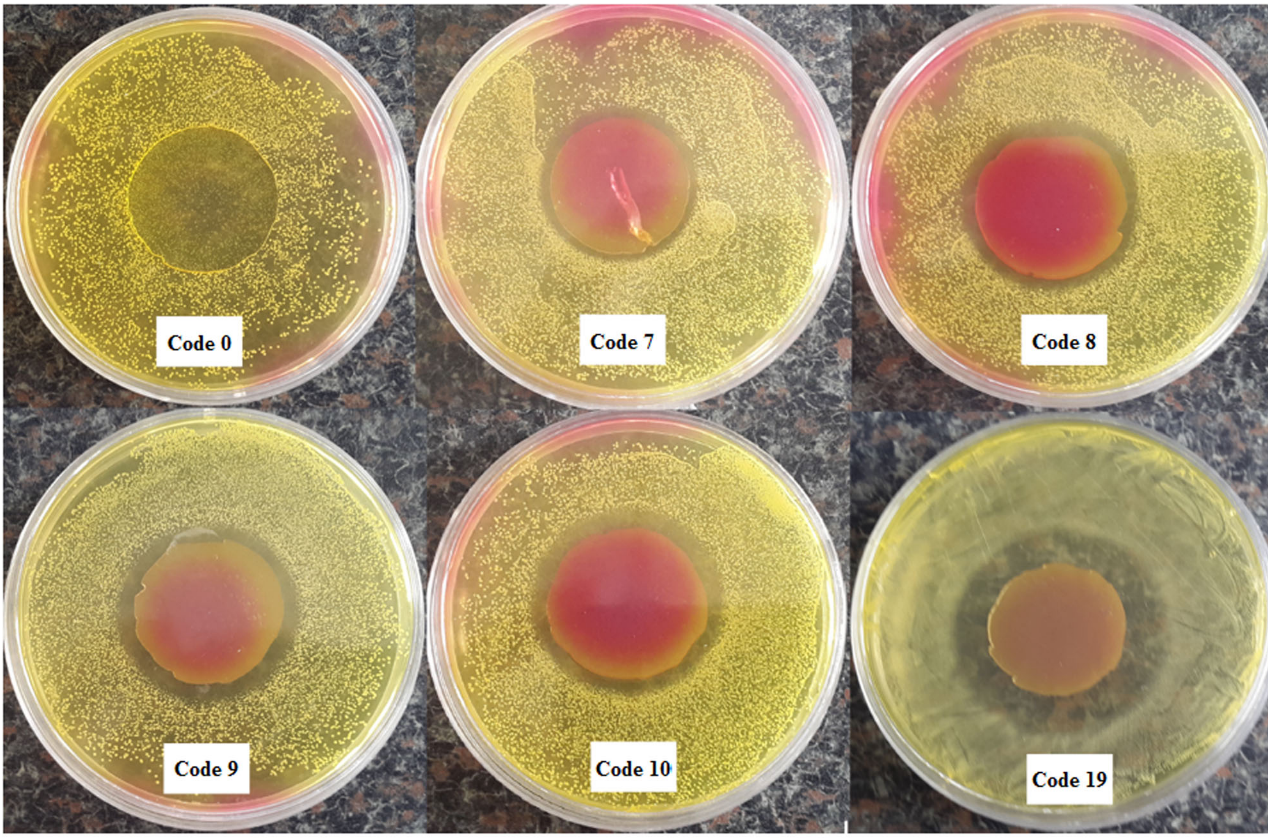


Figure 8–Changes in the inhibition zone in each plate containing pure CMC films and nanobiocomposites containing Ag nanoparticles at different levels of weight percent against *S. aureus*. Sample codes are described in Table 2.

UV absorption of nanobiocomposite films

Films made of CMC biopolymer have very high clarity that the radiation waves can easily pass through it both in the ultraviolet range and in the visible light range. By modifying these films by nanobiocomposite preparation using Ag, ZnO, and CuO nanoparticles, their transparency is reduced to a large extent, and the film's resistance to light waves increases. Figure 4 shows the effect of the introduction of three types of ZnO, CuO, and Ag nanoparticles into a CMC polymer matrix in the preparation of nanobiocomposites at different levels of weight percent on the absorption rate of ultraviolet radiation in the wavelength range of 200 to 800 nm. Films containing nanoparticles of ZnO and Ag in the investigated wavelength range peak have shown absorption. By increasing the weight of these nanoparticles in the nanobiocomposite film, the absorption rate of ultraviolet has been increased. The absorption rate of ultraviolet light in a nanobiocomposite film containing silver nanoparticles is in the wavelength range of 390 to 480 nm, with the highest absorption at 450 nm. A peak created for films containing ZnO nanoparticles is in the wavelength range of 350 to 380 nm. As you can see, nanobiocomposite film containing ZnO nanoparticles has the highest absorption in the entire wavelength range, which is one of the unique properties of this metal compound. The nanobiocomposite film containing the CuO in its entire wavelength range transmits ultraviolet radiation and does not absorb any radiation. For film containing Ag nanoparticles, the intensity of absorption is higher than that of ZnO nanoparticles, which suggests that the absorption capacity of silver nanoparticles is less than that of ZnO nanoparticles. It is clear that nanobiocomposite film containing ZnO alone has the best UV absorption performance, which is in consistent with the results of other researchers (Cárdenas, Diaz, Meléndrez, Cruzat, & Garcia Cancino, 2009; Li, Deng, Deng, Liu, & Li, 2010; Maity et al., 2012; Moura, Mattoso, & Zucolotto, 2012; Nafchi, Alias, Mahmud, & Robal, 2012; Pinto et al., 2012; Yu, Yang, Liu, & Ma, 2009). Figure 5 shows the effect of Ag, ZnO, and CuO incorporation into a CMC polymer matrix in the preparation of 2% nanoparticle nanobiocomposites on the absorption rate of ultraviolet radiation in the wavelength range of 200 to 800 nm. Films containing a combination of Ag and ZnO nanoparticles in the wavelength range investigated show the absorption peaks corresponding to the weight percent of the nanoparticles inside the matrix. By the inclusion of CuO as the third component of nanoparticles, composition of the nanoparticles and the intensity of peaks corresponding to the different Ag and ZnO nanoparticles has decreased, but the absorption peaks corresponding to these nanoparticles are observed after the addition of CuO nanoparticles.

WVP of nanobiocomposite films

One of the practical methods for improving the deterioration properties of biopolymers is the use of nanometric fillers within the biopolymer matrix or the so-called preparation of nanobiocomposites. The WVP for pure CMC films and CMC nanobiocomposite films containing Ag, ZnO, and CuO at RH of 97% and after 5 days was measured. Results of Ag, ZnO, and CuO nanoparticles alone are reported in Figure 6. The WVP for pure CMC film was 1.768×10^{-5} g/m·hr·Pa. By increasing the percentage of metal nanoparticles into the CMC matrix, the amount

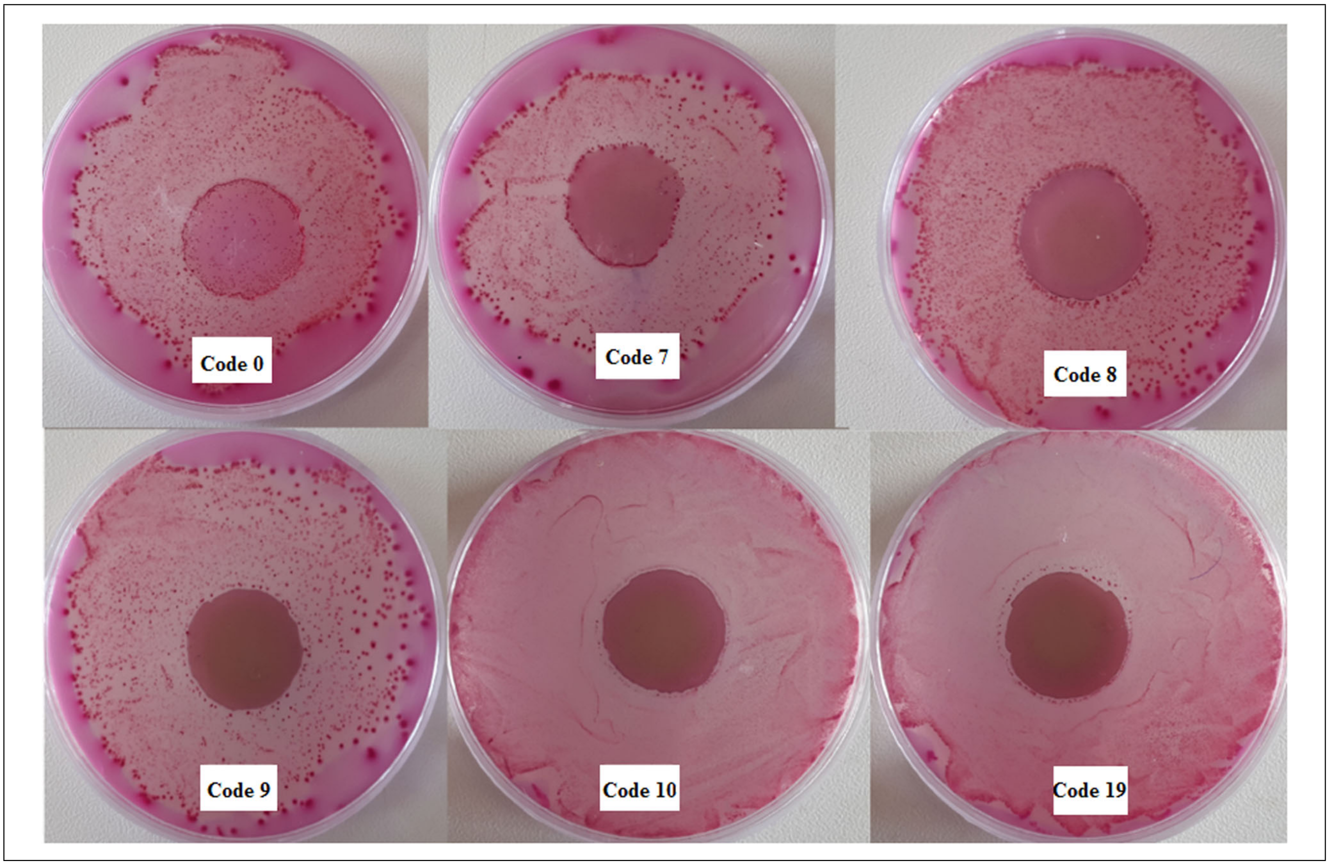


Figure 9–Changes in the inhibition zone in each plate containing pure CMC films and nanobiocomposites containing Ag nanoparticles at different levels of weight percent against *E. coli*. Sample codes are described in Table 2.

of WVP was gradually decreased. Reduction of WVP as a result of the introduction of nanoparticles into the biopolymer matrix can be attributed to several mechanisms: these particles, by being placed in empty spaces of the biopolymer matrix and creating zigzagging and polygonal paths, make the path for the movement of steam molecules more difficult and prolonged. Furthermore, cross-linking by metal nanoparticles reduces the mobility of the biopolymer chain and its structure is resistant to water vapor diffusion. Many researchers reported that the reduction of WVP was due to the placement of metal nanoparticles in the intermolecular space, filling the pore and free space of the biopolymer and also increasing the hydrophobicity of the polymer surface (Azeredo, 2009; Cárdenas et al., 2009; Nafchi et al., 2012). As the results of the permeability test for the nanobiocomposite films for all three types of metal nanoparticles in Figure 6 show, with the increase in the weight of nanoparticles within the biopolymer matrix, the WVP of the nanobiocomposites decreased and this property improved for these films. Among different amounts of weight percent of all three nanoparticles, metal silver exhibits better performance in reducing permeability for vapor. This fine performance of silver nanoparticles may be due to its better distribution in the CMC matrix compared to two other nanoparticles, providing strong interactions between the CMC chains and silver nanoparticles, as well as the filling of empty spaces between the chains. The SEM images also show this interaction in the smooth morphology of the surface of nanobiocomposite films containing silver nanoparticles. Figure 7 shows the results of the measurement of WVP of nanobiocomposite films containing a mixture of Ag, ZnO, and CuO nanoparticles at 2% wt.% under RH of 97% and after 5 days. As can be seen, films containing Ag, ZnO, and CuO nanoparticles at a 2% wt.% concentration had lower WVP values than those of the control (pure CMC film), as well as nanobiocomposite films containing single nanoparticles in the same. The level of weight percentage indicates that there is a coherent property of the composition of the nanoparticles in counteracting the passage of steam molecules from the inside of the biopolymer matrix. In other words, by using the combination of nanoparticles in their individual state within the biopolymer matrix, steam molecules must pass a longer and more complex pathway to pass the film, which reduces the transfer and penetration of steam molecules.

Antibacterial properties of nanobiocomposite films

Table 3 shows the results of pure CMC film samples and nanobiocomposites containing single metallic nanoparticles, ZnO, and CuO at different levels of weight percent in the microbial test. As is clear from this table, pure CMC film without metallic nanoparticles had no antibacterial properties. With the introduction of metallic nanoparticles into a CMC matrix in nanobiocomposite films alone, antimicrobial activity in films is observed with increasing inhibition area. It is clear that for all three types of single nanoparticles, by increasing the amount of metal nanoparticles, the inhibition area increased against both bacteria, and as a result, an antibacterial property was improved. Nanobiocomposite films containing nanoparticles of Ag and ZnO showed antibacterial properties against *S. aureus* and *E. coli*, whereas in the case of CuO, this property is only observed for *E. coli*; for both bacteria, the best antibacterial performance is observed in a nanobiocomposite film containing Ag nanoparticles. Table 3 also shows the results of pure CMC film and nanobiocomposites containing Ag, ZnO, and CuO nanoparticles at 2% wt.% in the microbial test. As shown in Table 4, nanobiocomposite films contain a combination of nanoparticles; with increasing silver concentration in the film, the diameter of the inhibition zone was increased. The best nanobiocomposite film for antibacterial properties for both types of bacteria was achieved for code 18, which contains the combination of Ag, ZnO, and CuO at weight percentages of 1.333, 0.333, and 0.333, respectively, whose antibacterial results are comparable to that of a nanobiocomposite film containing 2% wt.% of a single Ag nanoparticle. Figure 8 and 9 show the growth of colonies of *S. aureus* and *E. coli* in each plate containing pure CMC films and nanobiocomposites at different weight percentages of single Ag nanoparticles.

Conclusions

In this study, biodegradable CMC nanobiocomposite films containing Ag, ZnO, and CuO nanoparticles were prepared by solution casting method. SEM images showed a flat, smooth, and homogeneous breaking surface for a pure CMC film, which became morphologically rough and heterogeneous by the introduction of metallic nanoparticles into the CMC matrix. EDXA dot-mapping analysis showed a better and widespread distribution of metallic nanoparticles in the CMC-based nanobiocomposite films. Addition of single or combined Ag, ZnO, and CuO nanoparticles at 2% wt.% into the biopolymer matrix led to improvement of the mechanical properties of the resulting films. UV–Vis results showed that only films containing Ag and ZnO nanoparticles exhibited peak absorption in the wavelength of 200 to 800 nm. WVP gradually decreased with increasing in the percentage of metallic nanoparticles into the CMC matrix.

Nanobiocomposite films containing Ag and ZnO nanoparticles also exhibited antibacterial activity against *S. aureus* and *E. coli*, whereas films containing CuO nanoparticles only had antibacterial effect against *E. coli*. The best nanobiocomposite film for antibacterial properties for both types of bacteria was achieved for the film containing a combination of Ag, ZnO, and CuO at weight percentages of 1.333, 0.333, and 0.333, respectively (film No. 18).

Acknowledgments

This study was a part of MSc program carried out in Univ. of Tabriz (Tabriz, Iran). We thank Dr. S. H. Fasihnia for her assistance in performing microbial tests.

Author Contributions

Y. Ebrahimi performed the samples' synthesis, collected test data, and drafted the manuscript. S. J. Peighambardoust and S. H. Peighambardoust designed the study, interpreted the results, and revised the manuscript critically for important intellectual content. S. Zahed Karkaj sorted and analyzed data. All authors approve the final version of the manuscript and agree to be accountable for all aspects of the work.

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In this webinar, we will explore major pathogens in meat and the public health burden of foodborne illness outbreaks involving these pathogens, issues dealing with sampling and the microbiological testing of meat, and industry and regulatory approaches for assuring the safety and quality of meat and meat products. We will also discuss the 25th anniversary of HACCP regulation for meat and meat products, and its impact on meat safety.

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