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Development and Characterization of CMC/PVA Films Loaded with Zno-Nanoparticles for Antimicrobial Packaging Application

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ABSTRACT

Novel nanocomposite based on Polyvinyl alcohol (PVA), Carboxymethyl Cellulose (CMC) and Zinc Oxide Nanoparticles (ZnO-NPs), namely CMC/PVA/ZnO nanocomposites, was fabricated via solution casting methodology. ZnO-NPs were firstly prepared by hydrothermal method then different ratios of both CMC and PVA (50:50 and 60:40 v/v) were prepared to create novel blend, then ZnO-NPs was loadings with different concentrations to the prepared blend. The prepared ZnO-NPs were tested using X-Ray Diffraction pattern (XRD) and Transmission Electron Microscope (TEM). As well as CMC/PVA/ZnO nanocomposites were studied using Fourier Transform Infrared (FT-IR), TEM, XRD, UV/Vis spectroscopy and TGA. The films containing CMC/PVA in a ratio of (50:50 v/v) displays best morphological, thermal, mechanical antibacterial properties than films prepared using (60:40 v/v). The prepared nanocomposites displayed respectable antibacterial activity against gram positive (Staphylococcus aureus), gram negative (Pseudomonas aeruginosa, Escherichia coli) bacteria and fungi (Candida albicans). Furthermore, the fabricated nanocomposites can be used as good materials for packaging applications.

Keywords: PVA, CMC, Nanocomposite, ZnO-NPs, Antibacterial activity, Packaging applications

INTRODUCTION

In last few years, Nanotechnology can be used to meet the requirements of consumers in proving food's quality and in using antimicrobial agents to increase the shelf life of foods during storage and distribution. Food industries should choose the packaging materials that are suitable for their food products along with the benefits and disadvantages of these packaging materials [1]. Nanomaterials have broad range of applications in various fields, such as physics, chemistry, electronics, optics, materials science and biomedical sciences [2]. Therefore, inorganic/organic composite materials are prepared with many compositions where by combining organic and inorganic materials, the result composites may have advantages of both organic and inorganic materials, thus creating various usages in many applications. Among the many inorganic materials, zinc oxide [3].

Zinc Oxide is one of the most important types of nanoparticles that are used in improving the packaging materials properties due to its good antibacterial properties, high stability and photocatalytic activity. ZnO-NPs can be obtained by thermal method using zinc acetate [4]. Attributable to its good thermal and chemical stability and tunable optical and electrical properties [5], ZnO-NPs might be used in several applications for instance coatings for papers, pigments, optical material and cream lotions to protect against sunburn[6]. Furthermore, ZnO-NPs have various significant applications in biomedical field, as food additive, in catalysis and other important applications [7]. Constructively, Carboxymethyl Cellulose (CMC) is a natural biodegradable and biocompatible anionic polymer receiving from natural cellulose by chemical modification. Moreover, CMC could be used as potential fat replacers where it is non- digestible fibers [8]. Carboxymethyl cellulose may be coatings only and in blend with gamma irradiation was experienced for keeping the storage quality and improving shelf-life of plum. CMC-based coatings can be shared with gamma irradiation to achieve a synergistic influence concerning to storage quality and shelf-life of fresh fruits [9]. Xanthan gum (XG) and CMC can effect on the quality parameters of gluten-free flat bread, count on rice flour, rise in the concentration of CMC added bigger gas cells, leading to better crumb porosity[10]. Also, CMC is used for keeping moisture, and enhancing the mouth-feel and structural consistency of bakery products; it is also used in blending with other stabilizers and gums because of its high water-absorbing capacity [11]. Polyvinyl alcohol (PVA) as biodegradable polymer can be used in numerous applications attributable to its specific properties such as good fiber forming, good thermal and chemical stability along with high tensile and impact strengths. Alternatively, pure PVA has poor stability and swelling in water which decrease its use as a membrane in aqueous systems because the membrane swelling has a great influence on the separation performance of the membrane [12]. The good mechanical property, biodegradability and biocompatibilities of various polymer hydrogels are not enough to satisfy the customer needs for appropriate applications. The overcome the limitations of hydrogels in packaging materials application, by blending synthetic polymers such as PVA with some natural macromolecular materials such as CMC [13].

Zinc oxide nanoparticle is an ecofriendly and has concerned additional consideration in the last days owing to its good antibacterial properties, photocatalytic activity and high stability [14]. The mixture of ZnO-NPs as nanofillers to polymer matrix for preparation of nanocomposites might improve not only the prepared polymer nanocomposites properties such as, thermal, mechanical and barrier properties but also produce extra functions and applications in packaging for instance antimicrobial agent [7]. The antimicrobial activity is of ZnO-NPs is still under examination. The photocatalytic generation of hydrogen peroxide was recommended to be one of the primary mechanisms [15]. In addition, penetration of the cell covering and disorder of bacterial membrane upon contact with ZnO-NPs were also indicated to inhibit bacterial growth [16]. Conversely, the role of Zn^{2+} ion free from dissolution of ZnO is not clear, It may be binding to the membranes of microorganisms can delay the lag phase of the microbial growth cycle.

In this work we keen to prepare blend of CMC/PVA and then ZnO-NPs will added be different loadings%, then study the morphological, thermal and antibacterial properties of the prepared CMC/PVA/ZnO nanocomposite films.

MATERIALS AND METHODS

Materials

CMC (molecular weight 4.2×10^8, degree of substitution of 0.7) was purchased from Kelong Chemical Agent Factory, Chengdu, China. Zinc-acetate (Puriss, Reanal, Hungary) (Zn $(CH_3COO)_2$. $2H_2O$) was used to prepare ZnO. Sodium hydroxide (NaOH) was purchased from Acros. Poly(vinyl alcohol) was supplied from Sigma Aldrich. Distilled water was used in all preparation procedures. All chemicals and reagents are of analytical grade and used without any further purification.

Preparation of ZnO nanoparticles

We can prepare ZnO nanoparticles by the reaction of zinc acetate and a base in the alcohol medium. In this study, 3.942 g zinc acetate and 1.44 g NaOH were dissolved in 1 L ethanol and refluxed at 60°C for 1 h. The acetate group reacted with base and converted zinc acetate into zinc oxide. The prepared ZnO that is dispersed in alcohol medium was clear and transparent and was able to maintain stable for at least 2 weeks. After reaction, the zinc oxide that is dispersed in ethanol was mixed with DI-water for purification. ZnO particles were then separated from the dispersion supernatant by centrifugation at 7000 rpm for 5 min repeatedly. Finally the ZnO particles were dispersed in DI-water to obtain ZnO water dispersion.

Preparation of CMC/PVA/ZnO nanocomposites

The CMC/PVA//ZnO nanocomposites were prepared by solution casting method. Firstly, we prepared 5% CMC by dissolving 20gm CMC in 400ml DI-water as well as we prepare 5% PVA by the same method. Then, CMC/PVA blends were obtained by mixing the two polymer solutions by different ratio (50:50 v/v), (60:40 v/v) and (80:20 v/v). Then, prepared the composites by adding the different concentration of ZnO-NPs (2, 4 and 6 wt%) to different ratios of CMC/PVA blends (50:50 v/v), (60:40 v/v) and (80:20 v/v). The composites were prepared by gradually adding of the ZnO-NPs suspension (1 mg/ml in water) to the CMC/PVA solution and sonicating for 2 h at 25°C (Q500 Sonicator; sonication power, 500 W, frequency, 20 kHz and amplitude 50%). The homogeneous CMC/PVA/ZnO suspensions were poured into transparent glass petridish and left at room temperature for 72 h in order to evaporate the solvent and form the film.

Characterization

XRD patterns were obtained on a Bruker D8 advance diffractometer using CuKα radiation (λ=1.540 Å), operating at 40 kV and 40 mA. Scans were performed with a detector step size of 0.02° over an angular range of 2θ starting from 10 to 80°. The morphological studies were carried out using transmission electron microscopy (TEM) (JEM-1230). The chemistry of the hybrids was analyzed using a Fourier-transform infrared (FT-IR) spectrometer (Nicolet Magma 550 series II, Midac, USA) at wavelengths ranging from 4000-400 cm^{-1}.

Antimicrobial activity

Agar plate method has been recognized to estimate the antimicrobial activities of different textile treated samples [17,18]. Two bacterial test microbes; *Staphylococcus aureus* (Gram positive) and *Pseudomonas aeruginosa* (Gram negative); one yeast test microbe *Candida albicans* and one fungal test microbes, i.e., *Aspergillus niger* were selected to evaluate the antimicrobial activities. The bacterial and yeast test microbes were grown on a Nutrient Agar medium (NA) of the following ingredients (g/l): beef extract (3), peptone (10) and agar (20). On the other hand, the fungal test microbes were cultivated on Szapek-Dox agar medium of the following ingredients (g/l): Sucrose (30), $NaNO_3$ (3), $MgSO_4.7H_2O$ (0.5), KCl (0.5), $FeSO_4.7H_2O$ (0.055), K_2HPO_4 (1) and agar (20). The culture of each test microbe was diluted by distilled water (sterilized) to 10^7-10^8 colony forming units (CFU)/ml then 1 ml of each was used to inoculate 1 L Erlenmeyer flask containing 250 ml of solidified agar media [19]. These media were put onto previously sterilized Petri dishes (10 cm diameter having 25 ml of solidified media). Textile discs (10 mm Ø, Whatman No. 1 filter paper) loaded with 0.2 mg of each extract. The textile discs were placed on the surface agar plates seeded with test microbes and incubated for 24 h at the appropriate temperature of each test organism. Antimicrobial activities were recorded as the diameter of the clear zones (including the film itself) that appeared around the films [20].

RESULTS AND DISCUSSION

X-ray diffraction

Figure 1 represented the X-ray diffraction pattern (XRD) of CMC/PVA nanocomposites blend with different CMC: PVA (50:50 and 60/40) based on ZnO-NPs which added to the blend with different concentrations (2, 4 and 6% ZnO-NPs). The investigation was accomplished to evaluate the phase structure of the prepared CMC/PVA/ ZnO-NPs nanocomposites. Figure 1 displays the X-ray diffraction patterns of hybrid CMC/PVA/ZnO-NPs nanocomposite containing (50:50 v/v and 60/40 v/v) of CMC and PVA modified with different loadings of ZnO-NPs (2, 4 and 6%) the XRD of CMC/PVA blend there is no significant peak except peak at 2θ =19.6° that related to the crystalline cellulosic structure. While the prepared ZnO-NPs via environmental attitude, the pattern for ZnO-NPs indicated most characteristic peaks that corresponding to the planes (100) at 2θ=39.74°, (002) at 2θ=34.2°, (101) at 2θ=36.5°, (102) at 2θ=48.1°, (110) at 2θ=57.8°, (103) at 2θ=62° as well as (112) at 2θ=68.6°. The result obtained from XRD pattern revealed that the fabrication of zinc oxide nanoparticles very close to the packed hexagonal Wurtzite structure and the most of diffraction peaks agree with the stated JCPDS data.

Moreover, it has to be distinguished that the XRD of the prepared CMC/PVA/ZnO-NPs nanocomposites exhibited the presence of ZnO-NPs into the polymer matrix in all ZnO-NPs loadings as shown in (Figure 1a and 1b), confirmed the well formation of CMC/PVA/ZnO-NPs nanocomposites. Correspondingly, the intensity of ZnO-NPs peaks in the prepared nanocomposites increase by increasing the loadings of ZnO-NPs in the polymer matrix.

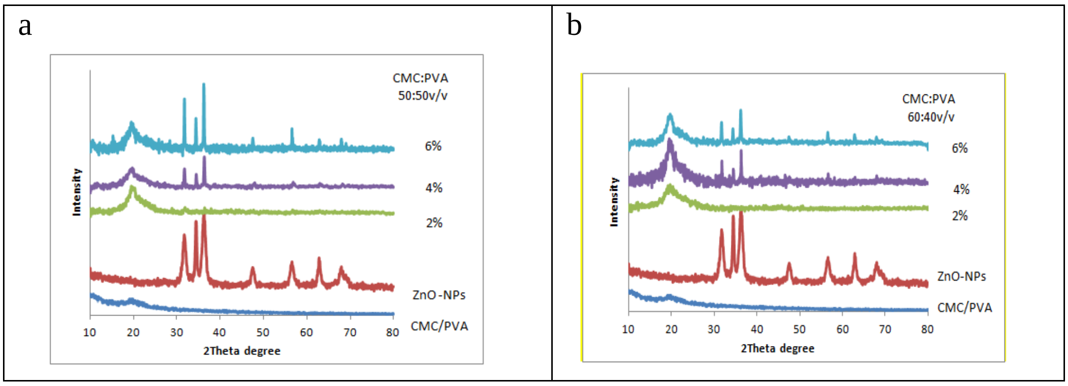

Figure 1: XRD patterns (a) for CMC/PVA blend, ZnO-NPs as well as CMC/PVA/ZnO nanocomposites loaded with 2%, 4% and 6 wt% of ZnO-NPs. for (a) CMC: PVA (50:50 v/v), (b) CMC: PVA (60:40 v/v)

FT-IR spectroscopy of the prepared nanocomposites

To prove possible intermolecular interactions between different components in the prepared nanocomposites system, FT-IR spectra of CMC/PVA blend and CMC/PVA/ZnO nanocomposite containing different concentrations of ZnO-NPs(2%, 4% and 6%) were recorded in the range 500-4000 cm^{-1} (Figure 2a and 2b).

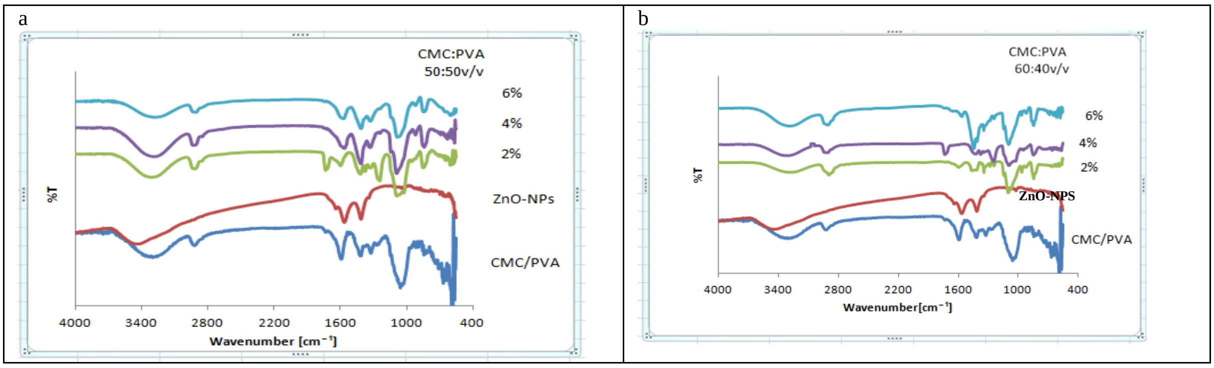

Figure 2: FT-IR spectra (a) CMC/PVA blend, ZnO-NPs and CMC/PVA/ZnO nanocomposite containing different concentrations of ZnO-NPs (2, 4 and 6%). for (a) CMC: PVA (50:50 v/v), (b) CMC: PVA (60:40 v/v)

Comparing the IR spectra of the prepared ZnO-NPs, CMC, PVA as well as the fabricate CMC/PVA/ZnO nanocomposites films, from IR spectra it could be observed that the exact peaks of ZnO-NPs, CMC, PVA and CMC/PVA blend, all peaks seemed in the spectrum of CMC/PVA/ZnO nanocomposite films, that suggests that there is no variation of the structure after compounding. CMC/PVA blend shows FT-IR absorption bands at 3285 and 2928 cm^{-1}, associated to the hydroxyl group (OH) stretching and group of (CH_2) asymmetric stretching related to CMC. Furthermore, the absorption bands concerning to amide I, amide II of C=O stretching vibrations, and N-H bending vibrations of NH_2 as well as and CH_2 flapping joined with OH group, this peaks at 1642 cm^{-1}, 1581 cm^{-1} and 1345 cm^{-1} respectively. The bands corresponding to OH groups shifted to 3310 cm^{-1} and changed into weaker signals for CH/CMC/ZnO nanocomposite films compared to CMC/PVA blend. The promising intermolecular interactions concerning these groups and nanoparticles influence the differences in the characteristic absorption bands.

TEM analysis

In order to investigate the morphology structure of the prepared ZnO-NPs as well as CMC/PVA/ZnO nanocomposites, TEM studies were done for the fabricated nanocomposite preparations. Figure 3 represented the TEM photographs of the prepared ZnO-NPs as well as CMC/PVA/ZnO nanocomposites. ZnO-NPs in single crystalline form were prepared through thermal method; using zinc acetate as precursor and high alkaline medium (0.5 M NaOH) for preparing ZnO-NPs at 160°C for 8 h using absolute ethanol. The data gained from TEM image approves the fabrication of ZnO-NPs, which is respectable agreement with XRD results (Figure 1). The prepared ZnO-NPs in the range of nanometer with an average size about 6 nm were dispersed in CMC/PVA blend, which lead to good result on the different properties of the prepared nanocomposites films such as; mechanical and biological properties. Moreover, Figure 3 revealed the TEM images of CH/CMC/ZnO nanocomposites containing two different ratios of CMC to PVA and various concentration of ZnO-NPs (2%, 4% and 6%) this image showed that the ZnO-NPs dispersed in the CMC/PVA matrix. It can be noticed that the dark areas relate to the crystalline ZnO-NPs whereas the bright areas indicate the CMC/PVA matrix, owing to the high electron density of the ZnO-NPs as shown in Figure 3.

UV/Vis spectroscopy of the prepared CMC/PVA/ZnO nanocomposites

The fabrication of metal nanoparticles can be recognized through the observation of the representative plasmon in UV/Vis spectra. Also, the particle size of the fabricated nanoparticles plays a significant role in varying the whole properties of the new nanocomposite materials.

Therefore, the size of prepared ZnO-NPs considers very important for discovering the new properties of the fabricated CMC/PVA/ZnO nanocomposites films.

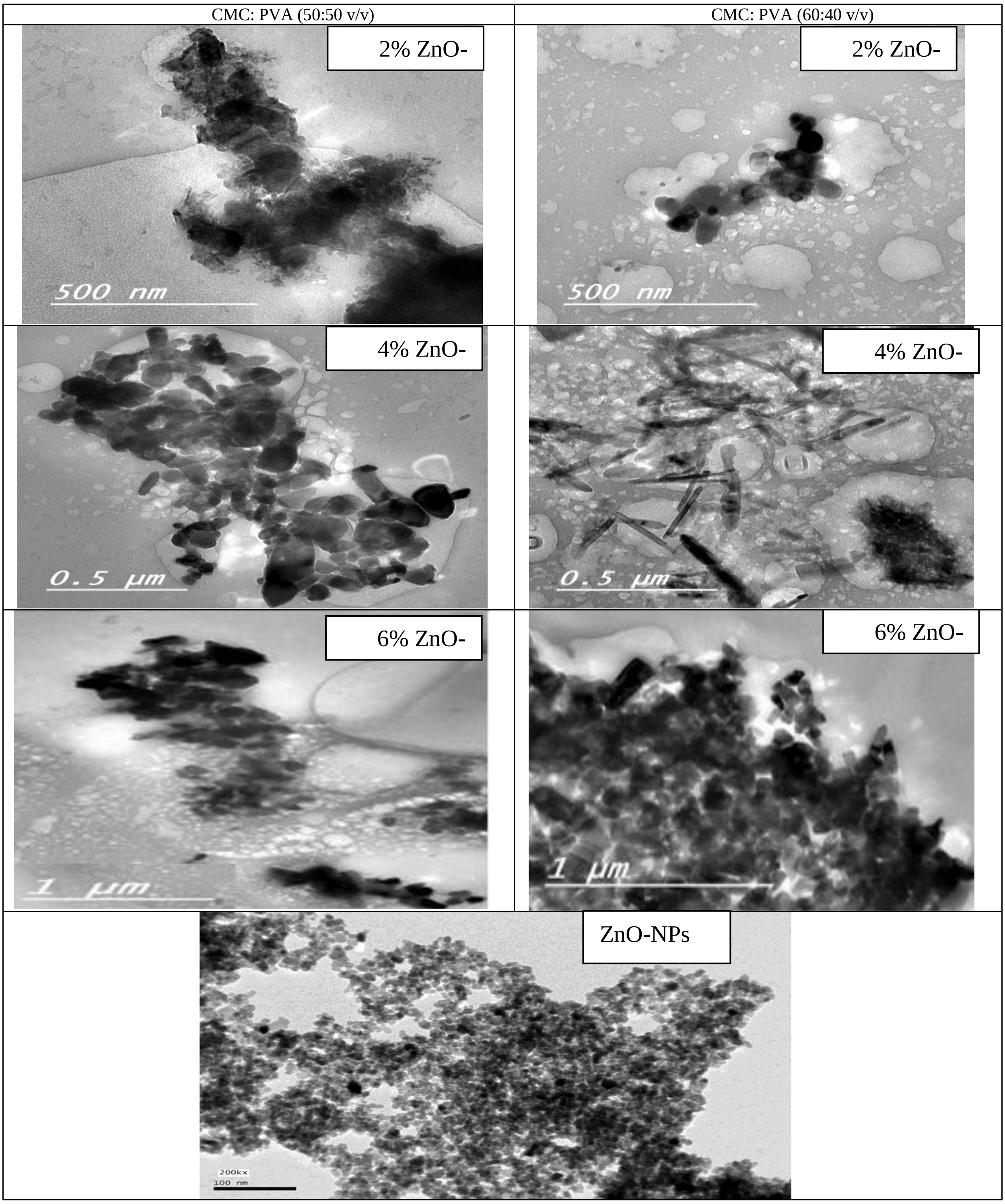

Figure 3: TEM image of ZnO-NPs as well as CMC/PVA/ZnO nanocomposites containing different concentrations of ZnO-NPs (2, 4 and 6%) for (a) CMC: PVA (50:50 v/v), (b) CMC: PVA (60: 40 v/v)

UV-Vis spectroscopy technique is extensively used to study the optical properties of the prepared nanoparticles. Figure 4 reveals the absorption spectrum of ZnO-NPs powder; it displays a strong absorption band at about 360 nm [13]. This evident that significant the sharp absorption of ZnO designates the monodisperse nature of the distribution of the prepared ZnO nanoparticle [14].

The UV/vis absorption spectra attainable in Figure 4 presenting a broad absorption band in the range of 300-500 nm which related to the excitation of the characteristic plasmon resonance band for zinc oxide nanoparticles. The increasing of concentrations of prepared ZnO-NPs in the CMC/PVA blend lead to increasing in the intensity of band around 360 in both CMC/PVA blend (50:50 and 60:40) in the absorption spectrum. Such statement is suggestive the founding of ZnO nanoparticles in the polymer matrix.

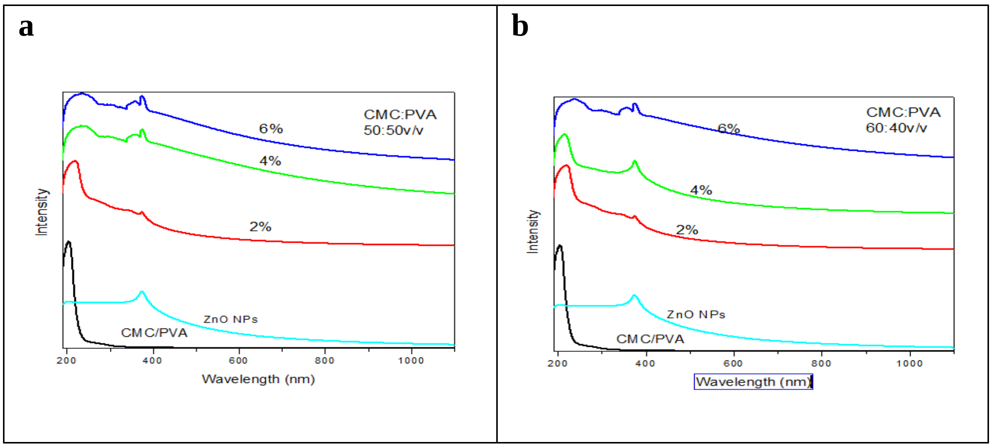

Figure 4: UV-Vis spectroscopy of ZnO-NPs as well as the CMC/PVA/ZnO nanocomposites with different concentrations of ZnO-NPs, (2, 4 and 6%) for (a) CMC: PVA (50:50 v/v), (b) CMC: PVA (60:40 v/v)

TGA analysis of the prepared CMC/PVA/ZnO nanocomposites

The thermal properties of the prepared nanocomposite materials are very essential and considered the most important characteristic of the key studies for packaging applications especially in food packaging. Thus, TGA is more important for construction and thermal stability of the fabricated (CMC/PVA/ZnO) nanocomposites as displayed in Figure 5. Figure 5 exhibited three different zones for each curve. The first minor loss in weight is because of the evaporation of water in polymer nanocomposites. The second the greatest break in each thermogram displays the onset of the decomposition process involving rapid loss in weight of the polymer nanocomposites due to the decomposition of the polymer backbone. The last step, the decomposition rate decreases steadily to a constant weight. CMC/PVA (50:50) blend was starting to decomposition at temperature (220°C) while after addition of ZnO-NPs with different loadings in the CMC/PVA/ZnO nanocomposite (2, 4 and 6% respectively) the thermal stability increased from 230°C to 280°C for 6% of ZnO-NPs compare with CMC/PVA blend. Additionally, a strong difference has been remarkable in TGA experiments in that CMC/PVA/ZnO nanocomposite when using CMC/PVA (60:40) the TGA displayed better thermal stability foe all samples containing different ratios of ZnO-NPs compare with CMC/PVA blend. Finally, the adding of ZnO-NPs to the CMC/PVA blend raises the thermal stability of the prepared CMC/PVA/ZnO nanocomposite.

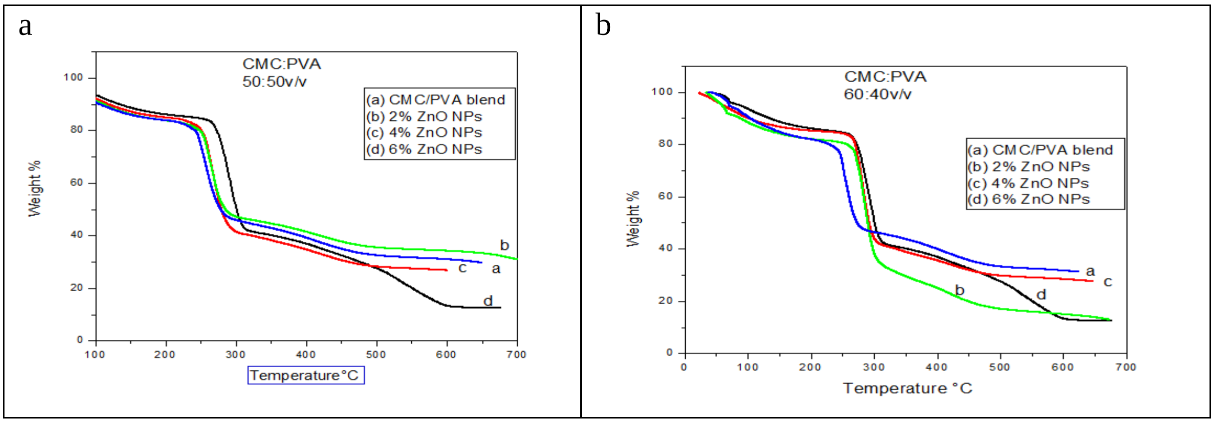

Figure 5: The thermal gravimetric curves of CMC/PVA blend as well as the CMC/PVA/ZnO nanocomposites with different concentrations of ZnO-NPs, (2, 4, and 6%) for (a) CMC: PVA (50:50 v/v), (b) CMC: PVA (60:40 v/v)

Antibacterial activities of the CMC/PVA/ZnO nanocomposites films

The evaluation of the antibacterial properties of the prepared CMC/PVA/ZnO nanocomposites films were carried out through agar plate method to determine the inhibition zone of the prepared films. Furthermore, these films were studied against gram positive (*S. aureus*), gram negative (*P. aeruginosa*) bacteria, fungi (*C. albicans*) and *Aspergillus niger*. This disc diffusion check has been done according to Youssef et al. [19]. Tables 1 symbolizes the clear inhibition zones diameter of the prepared nanocomposites films against descriptive strains of gram positive and gram negative bacteria and fungi. Also, Table 1 confirmed that the clear inhibition zone for all samples.

Figure 6 shows that the antibacterial activities of the prepared CMC/PVA/ZnO nanocomposites against the tested bacteria and fungi, in case of using CMC/PVA (50:50) there significant increasing in the inhibition zone with increasing the loadings of ZnO-NPs in the prepared nanocomposites films. Moreover, the clear inhibition zones of prepared nanocomposites films against demonstrative strains of gram positive and gram negative bacteria did not shown an significant change with raising the ratio of ZnO-NPs in case of the prepared nanocomposite films containing CMC/PVA (60:40) With different concentration of ZnO-NPs as shown in Figure 6. It is expected that the inhibitory effect of ZnO nanoparticles against microorganisms till now is not fully studied. It has been hypothetical that DNA misses its replication capability and cellular proteins become deactivated [19].

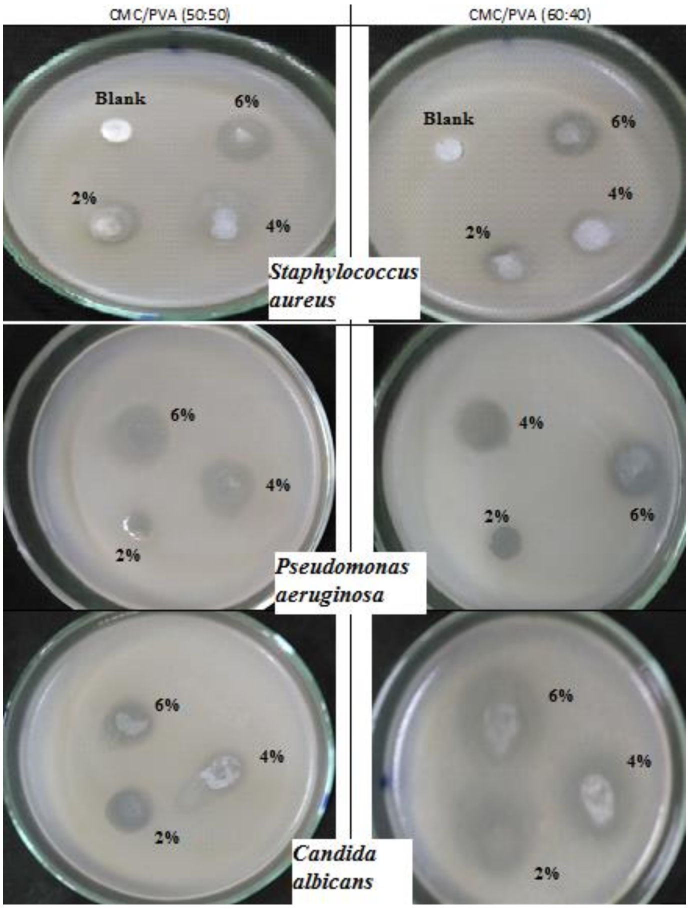

Figure 6: The antibacterial activity of the CMC/PVA/ZnO nanocomposite against gram positive (*Staphylococcus aureus*), gram negative (*Pseudomonas aeruginosa*) bacteria, fungi (*Candida albicans*)

Table 1: The antibacterial activity of the prepared CMC/PVA/ZnO nanocomposites

Clear zone of inhibition (f mm)						Sample
Aspergillus niger	***Candida albicans***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	**CMC:PVA Ratio**	**ZnO-NPs**	
0	0	0	0	0	0%	CMC/PVA
0	20	8	7	50:50:00	2%	CMC/PVA
9	22	17	18	50:50:00	4%	CMC/PVA
10	25	19	18	50:50:00	6%	CMC/PVA
0	0	0	0	60:40:00	0%	CMC/PVA
0	18	11	10	60:40:00	2%	CMC/PVA
8	19	11	11	60:40:00	4%	CMC/PVA
12	20	12	11	60:40:00	6%	CMC/PVA

CONCLUSION

The present work determines successful synthesis of CMC/PVA/ZnO nanocomposite based on different loadings of ZnO-NPs using solution casting method. Two different ratios of CMC/PVA were used in this study (50:50 and 60:40 v/v). The prepared nanocomposites films were evaluated using X-ray diffraction pattern (XRD) as well as the morphology was assessed via TEM. Moreover, the nanocomposites films were characterized using UV-Vis spectra it is obvious that the prepared ZnO-NPs existing in the prepared CMC/PVA/ZnO nanocomposites.

The fabricated nanocomposites films containing CMC/PVA in a ratio of (50:50 v/v) demonstrations good morphological, thermal, mechanical antibacterial properties than films prepared using (60:40 v/v). The prepared CMC/PVA/ZnO nanocomposites could be used as suitable materials for different packaging applications.

REFERENCES

[1] A.M. Youssef, S. M. EL-Sayed, H.S. EL-Sayed, H.H. Salama, A. Dufresne, *Carbohydr. Polym.*, **2016**, 151, 9-19.
[2] A.A. Hebeish, M.H. El-Rafie, F.A. Abdel-Mohdy, E.S. Abdel-Halim, H.E. Emam, *Carbohydr. Polym.*, **2010**, 82, 933-941.
[3] J.H. Chen, C.Y. Cheng, W.Y. Chiu, C.F. Lee, N.Y. Liang, *Eur. Polym. J*., **2008**, 44, 3271-3279.
[4] H. Li, J. Wang, H. Liu, H. Zhang, X.Li, *J. Cryst. Growth*., **2005**, 275, e943-e946.
[5] N. Tripathi, S. Rath, *Materials Characterization.*, **2013**, 86, 263-269.
[6] Y.T. Prabhu, K. Venkateswara Rao, V. Sesha Sai Kumar, B. Siva Kumari, *Advances in Nanoparticles.*, **2013**, 2, 45-50.
[7] A.M. Youssef, H. Abou-Yousef, S.M. El-Sayed, S. Kamel, *Int. J. Biol. Macromol*., **2015**, 76, 25-32.
[8] G. Monika, S. Valeriehuh, J. Weiss, *Food Hydrocolloids*., **2015**, 45, 236-246.
[9] P.R. Hussain, P. Suradkar, A.M. Wani, M.A. Dar, *Radiation Physics and Chemistry*., **2015**, 107, 136-148.
[10] M. Mohammadi, N. Sadeghnia, M.H. Azizi, T.R. Neyestani, A. M. Mortazavian, *J. Industr. Eng. Chem*., **2014**, 20, 1812-1818.
[11] Z. Kohajdova, J. Karovicˇova, *Chem. Pap*., **2009**, 63, 26.
[12] E. Karimi, A. Raisi, A. Aroujalian, *Polymer*., **2016**, 99, 642-653.
[13] F. Wang, Y. Wen, T. Bai, *Mater. Sci. Eng. C.,* **2016**, 69, 268-275.
[14] Y.D. Jin, J.P. Yang, P.L. Heremans, *Chem. Physics Lette.*, **2000**, 5-6, 387-392.
[15] Z.L. Wang, *J. Phys: Condensed Matter*., **2004**, 16, R829-R858.
[16] J. Sawai, *J. Microbiol. Methods*., **2003**, 54, 177-182.
[17] C.H. Collins, P.M. Lyne, Butterworth & Co. Pub. Ltd., London, UK & Toronto, Canada, **1985**, 167-181.
[18] M.S. Abdel-Aziz, A.M. Hezma, *Technol. Eng*., **2013**, 52(13), 1503-1509.
[19] A.M. Youssef, M.S. Abdel-Aziz, S.M. El-Sayed, *Int. J. Biol. Macromol.*, **2014**, 69, 185-191.
[20] M.S. Abdel-Aziz, K.S. Abou-El-Sherbini, E.M.A. Hamzawy, M.H.A. Amr, S. El-Dafrawy, *Appl. Biochem. Biotectnol*., **2015**, 176, 2225-2241.