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Preparation and characterization of chitosan nanocomposite based on nanoscale silver and nanomontmorillonite

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ABSTRACT

The chitosan nanocomposites were rapidly prepared by simple solution method. This biopolymer matrix was modified by prepared nanoscale silver (Ag) using *in situ* synthesis from precursor and nanomontmorillonite (NMMT). Moreover, the samples were characterized by fourier transform infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA), field emission scanning electron microscopy (FESEM), and energy dispersive x-ray spectroscopy (EDX). Moreover, the water vapor properties (WVP) of nanocomposites were investigated using gravimetric standard. In addition, the antibacterial activity of nanocomposite was measured by the well diffusion method on Muller–Hinton Agar against *Escherichia coli* (*E. coli*) by zone inhibition. Finally, based on the obtained results, the nanocomposite can have a good candidate for different applications and food packaging industry.

1. Introduction

Today, nanotechnology has expanded in various fields duo to the potential of application benefits in the world [1]. Nanomaterials have size of 1-100 nm that showed unique properties because of small size and high surface area [2]. In recent years, population growth was resulted in the extension of packaging for food source protection [3]. Since, nanotechnology can be affected because of the wide application in production, processing, storage,

* Corresponding Author: Negar Motakef-Kazemi Email: motakef@iaups.ac.ir https://doi.org/10.24200/amecj.v2.i2.56 packaging, and transport of food products with environment protection and earth resources [4]. The main factor is included a lot of effort to produce functional materials, food processing, product development, methods, and tools design [5]. The application of nanocomposite is expanded because of its unique properties in food packaging [6,7]. The nanocomposites can control the permeability of polymer and increase shelf life to improve the efficiency of packaging materials [8]. In packaging industry, the use of biodegradable material can reduce the waste and results to consume the less material precursor [9].

The nanomaterials have been developed the

research work in food products [10]. Recently, biodegradable nanocomposite has been used to investigate barrier materials and antimicrobials in packaging [11]. Moreover, nanocomposite based on biopolymer can be named such as starch [12,13], cellulose [14], and chitosan (CS) [15,16] for food packaging. Moreover, chitosan is derived from chitin, and its use has expanded as biomaterials [17]. The chitosan has several excellent properties such as biodegradability, non-toxicity, and antimicrobial properties which investigated in food packaging widely [18-20]. The polymer nanocomposite included the polymer as matrix phase and nanomaterial as filler phase to improvement of physical and mechanical properties [21]. In addition, the use of nanocomposites can enhance the applications of different properties for nanomaterials in food packaging technology [22]. Moreover, silver nanoparticles are well known as antimicrobial agents in curative and preventive health care with low toxicity for humans [23,24]. Clay filler has received significant attention because of suitable dispersion, thermal stability, and barrier properties in polymer nanocomposites [25]. In the present study, chitosan nanocomposite based on nanoscale silver and nanomontmorillonite has been prepared by a simple method using *in situ* synthesis of nanoscale silver for food packaging. The aim of this work is to study antibacterial activity against Escherichia coli and barrier for water vapor permeability of this nanocomposite.

2. Experimental procedure

2.1. Materials

All chemicals used were analytical grade. Chitosan was obtained from Sigma Aldrich with medium molecular weight. Glacial acetic acid, trisodium citrate and silver nitrate (AgNO₃) were obtained from Merck. Ultra-pure water was used for the preparation of all reagents solutions. Moreover, the modified montmorillonite clay was obtained from Nano Pasargad Novin Company. The test strains, *Escherichia coli* ATCC 1399, were procured from Islamic Azad University.

2.2. Methods

2.2.1. Preparation of nanoscale Ag

The nanoscale silver was prepared by reducing silver nitrate using trisodium citrate. $AgNO_3$ solution (2 M) was added to the 1% acetic acid solution. Then trisodium citrate solution (4 M) was added to the resulting solution and stirred for 2 h. The nanoscale silver was centrifuged at 10,000 rpm for 10 min and dried at room temperature for 48 h [26].

2.2.2. Preparation of chitosan–nanoscale Ag nanocomposite

Chitosan aqueous solution of 2 wt.% was prepared by dissolving chitosan powder (2 g) in 100 mL of 1% (v/v) acetic acid. Then AgNO₃ solution (2 M) and trisodium citrate (4 M) were added to the chitosan solution and stirred for 2 h at room temperature. The preparation of chitosan–nanoscale Ag nanocomposite was done by in situ synthesis of nanoscale silver. The mixture solution was cast onto glass plates and dried at room temperature for 48 h [26-27].

2.2.3. Preparation of chitosan–NMMT nanocomposite

Chitosan aqueous solution of 2 wt.% was prepared in acetic acid solution (1% v/v). The nanomontmorillonite was prepared by dispersing of montmorillonite clay into 10 mL of 1% acetic acid solution and vigorously stirring for 24 h. The nanomontmorillonite solution (1% and 3 wt.%) was added slowly to chitosan solution and stirred continuously for 2 h. Then the mixture was cast onto glass plates and dried at room temperature for 72 h [26-27].

2.2.4. Preparation of chitosan–nanoscale Ag– NMMT nanocomposite

Chitosan aqueous solution of 2 wt.% was prepared by 1% (v/v) acetic acid. Then AgNO₃ solution (2 M), trisodium citrate (4 M), and nanomontmorillonite solution (1% and 3% wt) were added into the chitosan solution and stirred for 2 h at room temperature. Then the mixture was cast onto glass plates and dried at room temperature for 72 h [26].

2.3. Characterization of samples

The samples were characterized by Fourier transform infrared spectroscopy, thermogravimetric analysis, field emission scanning electron microscopy, and energy-dispersive x-ray spectroscopy. FTIR spectra were obtained using a FTIR spectrophotometer (Shimadzu Co.) in the range of 400-4000 cm⁻¹ at a resolution of 4 cm⁻¹ in a KBr matrix. The thermogravimetric analysis was measured by a TGA-SF1 (Mettler Co.) that carried out to 800 °C at the heating rate of 20 °C per min under nitrogen atmosphere. The morphologies of the samples were analyzed on a MIRA3 TeScan LMU (TeScan Co.) FESEM at 5 kV. Afterwards, the specimens were coated with a thin conductive gold layer before observation. The EDX analysis was measured by usingASK SEM-CL view VIS (Oxford instruments Co.) The antimicrobial activity was investigated for pure chitosan, montmorillonite, and chitosan-based nanocomposites by measurement of the minimum inhibition concentration (MIC) against E. coli as gram-negative bacteria. The cells of E. coli were cultivated on Mueller-Hinton Agar and incubated at 37°C for 1 day. The samples were systematically diluted from 0.02% (w/v) to 0.000625% (w/v) to determine of the MIC values.

The WVP of films was determined using gravimetric standard ASTM E96-05 [28]. Glass bottles were used to perform the test with a diameter of 20 mm and depth of 45 mm. The bottles were filled with 3 g of CaCl₂ for maintaining a relative humidity (0% RH) and covered with the film specimen. The result bottles were placed in a container containing K_2SO_4 super saturated solution (97% RH) at 25 °C. Then the bottles were weighed ten times at 3 h intervals. Afterwards, water vapor transmission rate (WVTR) was determined from slope of mass change of bottle versus time curve divided by area of glass bottle mouth (m²). Then the WVP of film was calculated using Eq. 1:

 $WVP = WVTR \times L / \Delta P \quad (1)$

where WVTR is water vapor transmission rate

(g/m²s) through film, L is the mean thickness of film (m), and ΔP is partial water vapor pressure difference (Pa) across film [21].

3. Results and Discussion 3.1. FTIR

The FTIR spectra are shown for CS, nanoscale Ag, NMMT, CS-nanoscale Ag nanocomposite, CS-NMMT nanocomposite and CS-nanoscale Ag-NMMT nanocomposite in Fig. 1. The spectrum of chitosan (Fig. 1a) shows the band at 1647 cm⁻¹ that corresponds to the amide function due to acetylated amine, whereas the band at 1600 cm⁻¹ is corresponded to the free amine I function due to deacetylated amine. The broad band at 3400-3500 cm⁻¹ is assigned with the overlapping O-H stretching bands. The N-H bending, C-H bending, and C-O stretching are shown at 1600cm⁻¹, 1382 cm⁻¹ and 1087 cm⁻¹ respectively. The results corresponded to the results [27, 29]. The FTIR spectrum of NMMT (Fig. 1b) is shown the vibration bands at 3624 cm^{-1} for O-H, at 3425 cm⁻¹ due to interlayered O-H, at 1641 cm⁻¹ for H-O-H bending, at 1035 and 914 cm⁻¹ for Si-O stretching, and at 528 and 468 cm^{-1} for Si-O bending. These characteristic peaks have shifted to lower frequencies in nanocomposites which recommend some interaction between functional groups of chitosan and NMMT. The results are corresponded to the reported results [30, 31]. The FTIR spectra of CS-NMMT nanocomposite and CS-nanoscale Ag-NMMT nanocomposite (Fig.1c and 1d) are shown the combination of the characteristic absorption bands due to nanoscale Ag, NMMT and amine groups of chitosan. The overlapping peak of O-H and N-H shows more broadening at 3103-3392 cm⁻¹ in pure chitosan. The bands of amine groups are shifted to 1616 and 1668 cm⁻¹ in the CS-NMMT and CS-NMMT -nanoscale Ag nanocomposites. This shift corresponds to deformation and vibration of chitosan amine groups. The characteristic bands of clay are all shifted to lower absorption wavenumber from 500 to 1035 cm⁻¹ as shown on the figure. These observations are confirmed the electrostatic interaction of chitosan's NH₂⁺ groups with the negatively charged silicate

layer of clay in both composites. The spectrum of CS-nanoscale Ag nanocomposites is investigated (Fig.1 e). The omission of existing shoulderin 1548 cm⁻¹ is related to NH bending vibration bond that shows interaction between chitosan and nanoscale Ag that corresponds to the results [27]. The FTIR spectra of nanoscale Ag (Fig.1f) are shown the peaks at 1643 cm⁻¹, 1578 cm⁻¹, 1392 cm⁻¹ and 1278 cm⁻¹ that corresponded to the results [31]. Based on FTIR results, the qualitative change is confirmed for nanocomposite formation.

3.2. TGA

The weight-loss curve was obtained by heating of samples and determined thermal stability analysis as the function of temperature. The TGA analysis is shown for CS-NMMT nanocomposite that two weight loss regions were observed (Fig. 2. a), about 100°C (related to loss of water), and about 300°C (related to degradation of chitosan), at the result the remained weight perent was related to NMMT. The TGA analysis is shown for CS-nanoscale Ag



Fig. 1. Transmission FTIR spectra of (a) pure CS, (b) NMMT, (c) CS-NMMT nanocomposite, (d) CS-nanoscale Ag-NMMT nanocomposite, (e) CS-nanoscale Ag nanocomposite and (f) nanoscale Ag.

nanocomposite that two weight loss regions were observed (Fig. 2. b), about 100°C (related to loss of water), and about 300 °C (related to degradation of chitosan), at the result the remained weight perent was related to silver. The TGA analysis is shown for CS-nanoscale Ag-NMMT nanocomposite that two weight loss regions were observed (Fig. 2. c), about 100°C (related to loss of water), and about 300°C (related to degradation of chitosan), at the result the remained weight perent was related to NMMT and silver. Based on TGA results, thermal stability of nanocomposite which are as follows: CS-NMMT > CS-nanoscale Ag- NMMT > CS-nanoscale Ag that the presence of NMMT and nanoscale Ag have been caused an increase and decrease in this property respectively. CS-nanoscale Ag- NMMT was average thermal stability because of the opposite effects of two fillers. The TGA results are comparable to those of previous researchers [32].

3.3. FESEM

The size and morphology structures of samples were studied using FESEM that shown nanoscale Ag, NMMT, CS-nanoscale Ag nanocomposite, CS-NMMT nanocomposite, CS-nanoscale Ag-NMMT nanocomposite in Figure 3. Moreover, the NMMT has shaped sheet-like layer silicates and that included a two-dimensional layer with thickness of about 1 nm and length and width of about 300 nm to several microns (Fig. 3a). The morphology of nanoscale Ag has been nanorod arrays with the mean diameter of about 40 nm and the length of



Fig. 2. The TGA of (a) CS-NMMT nanocomposite, (b) CS-nanoscale Ag nanocomposite, and (c) CS-nanoscale Ag-NMMT nanocomposite.

about 200 nm (Fig. 3b). The morphology of CSnanoscale Ag has been nanorod arrays with the mean diameter of about 70 nm and the length of about 250 nm (Fig. 3c) that slightly increased in size due to the presence of chitosan. Moreover, the CS-NMMT nanocomposite is shown the palte-like structure with thickness of 30 nm and length and width of about 500 nm (Fig. 3d). The CS-nanoscale Ag-NMMT nanocomposite is shown nanoparticle morphology for nanoscale Ag with size of about 40 nm and palte-like structure for NMMT with diameter of 40 nm and length and width of 200 nm (Fig. 3e). These images are represented the uniform structure and complete distribution in the matrix of chitosan polymer. Also, the nanoscale silver morphology is observed nanoparticle instead of nanorod for chitosan nanocomposite in the presence of NMMT. In fact, NMMT is acted as capping agent and controlled crystal growth.



Fig. 3. The SEM of (a) NMMT, (b) nanoscale Ag, (c) CSnanoscale Ag nanocomposite, (d) CS-NMMT nanocomposite and (e) CS-nanoscale Ag-NMMT nanocomposite.

3.4. EDX

The EDX analysis is shown for nanoscale Ag, Cs-nanoscale Ag nanocomposite, (c) CS-NMMT nanocomposite and CS-nanoscale Ag-NMMT nanocomposite in figure 4. The intense signal has been at 3 keV that confirmed the presence of nanoscale silver (Fig. 4a). The EDX analysis of CSnanoscale Ag nanocomposite has been the signal of nanoscale silver at 3 keV and appeared the other signal of C, O, and N due to chitosan (Fig. 4b). Based on the result is confirmed the presence of CS and nanoscale silver. The EDX analysis of CS-NMMT has been the signal of clay for Al and Si and appeared the other signal of C, O, and N due to chitosan (Fig. 4c). The EDX analysis of CS-nanoscale Ag-NMMT nanocomposite has indicated the presence of silver, chitosan and clay in the nanocomposite (Fig. 4d).

3.5. WVP tests

WVP test is used to study water vapor barrier properties of packaging films and investigated the inhibition of the humidity exchange between food and environment. The WVP of samples was determined by the gravimetric method using WVP (Table 1). The chitosan film was showed the reduction in WVP permeability. The increase and decrease in WVP permeability were observed because of the addition of nanoscale Ag and NMMT respectively into chitosan nanocomposite films. The nanoscale Ag can form the hydrogen bond and resulted to the increase passage while NMMT with high aspect ratio can disperse uniformly in polymer matrix and lead to prevent permeability.

3.6. Antibacterial activity

The antibacterial activity of samples was tested against E. coli bacteria using the well diffusion method on Muller–Hinton Agar. All tests were done in triplicate. The antibacterial tests were summarized in Table 2 that observed the effect of chitosan and nanoscale Ag while NMMT has not exhibited any effect. The inhibition zone was the same for nanoscale Ag and chitosan–nanoscale Ag and the effect of nanoscale Ag has been



Fig. 4. EDX analysis of (a) nanoscale Ag, (b) CS-nanoscale Ag nanocomposite, (c) CS-NMMT nanocomposite and (d) CS-nanoscale Ag-NMMT nanocomposite.

Table 1.	WVP	test for	samp	les
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Sample	Sample	WVP (g/(m s Pa))
1	CS solution 2% wt	1.92×10^{-10}
2	CS-Silver nancomposite	4.31×10^{-10}
3	CS-MMT nancomposite 3%	1.63×10^{-10}
4	CS-MMT nancomposite 5%	1.17×10^{-10}
5	CS- Silver -MMT nancomposite 3%	$4.78 imes 10^{-10}$
6	CS- Silver -MMT nancomposite 5%	3.25×10^{-10}

evident apparent in comparison with chitosan. The minimum inhibitory concentration was 0.005 mol/L^{-1} for chitosan–nanoscale Ag composite. Antibacterial activity results are comparable to those of previous researchers [33].

4. Conclusions

The chitosan nanocomposites were successfully prepared from NMMT and *in situ* synthesis of nanoscale silver using the reduction of AgNO₃ by trisodium citrate. Based on FESEM results, *in situ* synthesis of nanoscale Ag was formed silver nanoparticle in chitosan nanocomposite duo to the

presence of NMMT although the silver nanorod was observed by reduction of silver precursor. Moreover, the TGA analysis was shown the increase in thermal stability duo to the presence of NMMT. The FTIR and EDS of nanocomposites were confirmed the presence of chitosan and silver. Finally, the effective role of NMMT and nanoscale Ag were observed in the decrease of WVP permeability and increase in antibacterial activity against *E. coli* respectively. Furthermore, based on these results chitosan–nanoscale Ag–NMMT nanocomposite can have a good potential for packaging.

Sample	Sample	Inhibition zone (mm)
1	CS solution 2% wt	9
2	CS-Silver nancomposite	12
3	Silver nanoparticles	12
4	CS-MMT nancomposite	9
5	CS- Silver -MMT nancomposite	12

Table 2. Inhibition zone of samples.

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