Journal of Materials Science and Engineering with Advanced Technology
Volume 7, Number 2, 2013, Pages 93-123

CARBOXYMETHYLCHITOSAN/VISCOSE BLENDED FILMS: PREPARATION, CHARACTERIZATION AND ANTIBACTERIAL PROPERTIES

N. Y. ABOU-ZIED[1], A. I. WALY[1], N. G. KANDILE[2], A. A. RUSHDY[3], MANAL A. EL-SHEIKH[1] and H. M. IBRAHIM[1]

[1]Textile Research Division
National Research Center
Dokki, 12311, Cairo
Egypt
e-mail: hmaibrahim@gmail.com

[2]Department of Chemistry
Faculty of Girls for Arts
Science and Education
Ain Shams University
Heliopolis, 11757, Cairo
Egypt

[3]Department of Botany
Faculty of Girls for Arts
Science and Education
Ain Shams University
Heliopolis, 11757, Cairo
Egypt

Keywords and phrases: cellulose xanthate, viscose, carboxymethylchitosan, polymer blend film, antibacterial properties.

Received November 10, 2012

Abstract

Modification of chitosan (CS) using monochloroacetic acid in the presence of sodium hydroxide under different reaction conditions were studied to determine the optimum conditions for preparing the O-carboxymethylchitosan (O-CMCS). The prepared O-CMCS was characterized by its nitrogen content, degree of substitution and its solubility in different solvent or water under different pH values. Evaluation of the antibacterial activity of CS and O-CMCS expressed in MIC and MBC against *E. coli as* Gram (–ve) bacteria and *S. aureus* as Gram (+ve) bacteria via broth tube dilution method was investigated.

O-CMCS /viscose blended films were prepared by mixing O-CMCS and viscose in water as co-solvent, and regenerated in coagulation bath mentioned in the experimental part. The obtained O-CMCS/viscose blended films were characterized via its nitrogen content, tensile strength, elongation at break, Fourier transform infrared spectroscopy (FT-IR), and thermal gravimetric analysis (TGA). Evaluation of the antibacterial activity of the O-CMCS /viscose blended films, against Gram (–ve) bacteria and Gram (+ve) bacteria was measured via both disk diffusion method and bacterial count methods. These blended films show good antibacterial effects towards both Gram (+ve) and Gram (–ve) bacteria. The antibacterial activity of the blended films increased and the tensile strength decrease as the O-CMCS percent increase in the blended film.

1. Introduction

Cellulose is poly-β-1,4-D-glucopyranose and it is biodegradable, nontoxic, biocompatible, hydrophilic, safe, has high moisture-retentivity, and chiral structure. Therefore, making use of cellulose to produce various products not only can protect the environment from pollution but also can save limited oil resources because of its biodegradability and potential to substitute for some petrochemicals, and also it has many uses as emulsifier, stabilizer, dispersing agent, thickener, and gelling agent, especially for the preparation of textile. However, cellulose has not reached its potential application in many areas because of its infusibility and insolubility (Isogai & Atalla [15]; Liu et al. [21]; Wub et al. [39]; and Zheng et al. [46]).

Chitosan is a copolymer of glucosamine and N-acetyl glucosamine units linked by 1,4-D-glucosidic bonds (Isogai & Atalla [15]; Urreaga & Orden [35]). It has good biocompatibility, biodegradability, non toxic, and various biofunctionalities including homeostatic, immunity enhancing, wound healing, antibacterial, and antifungal activities with a broad spectra and high killing rate, so that it is widely used in medicine, nutrition, cosmetics, several pharmaceutical and biomedical application fields, textile finishes, artificial skin, ophthalmology, membranes, hollow fibers, drug delivery system, and cell-stimulating materials etc. (Basavaraju et al. [2]; Liu et al. [21]; Rogovinaa et al. [26]; Shih et al. [29]; Twu et al. [34]; Urreaga & Orden [35]; Wub et al. [39]; Zheng et al. [46]; Zhou et al. [51]; and Zhuang & Liu [49]). On the other hand, poor solubility of chitosan in some common solvents, e.g., water, alkali, and organic solvents, limits its applications. To overcome this problem, chemical modification of chitosan is required and lots of derivatives have been synthesized. Among these, O-carboxymethylchitosan (O-CMCS), which is a kind of derivative introducing a -CH_2COOH group at C-6 position, shows stronger antibacterial activities and better solubility in water than chitosan (Angewandte [1] and Zheng et al. [46]). O-CMCS is not only soluble in water, but has unique chemical, physical, and biological properties such as high viscosity, large hydrodynamic volume, low toxicity, biocompatibility and film, gel-forming capabilities, all of which make it an attractive option in connection with its use in food products and cosmetics (Chen et al. [5]).

Introduction of some amino groups into a cellulosic system is important to facilitate chemical modification. However, it is generally difficult to introduce sufficient amounts of amino groups into cellulose by simple and efficient reactions without significant degradation of the cellulose chains. Chitosan has received considerable attention as an amine-containing polymer (Isogai & Atalla [15]). Cellulose and chitosan differ only in that the latter has the amino group instead of the hydroxyl group at C-2 of the glucopyranose ring. This similarity in primary structures suggests that the secondary structures and patterns of aggregation may also be sufficiently similar to facilitate the formation of homogeneous blends of the two polymers (Isogai & Atalla [15]; Liu et al. [21]; Twu et al. [34]; and Zheng et al. [46]).

Blending of polymers, which results in preparation of new materials with improved physicochemical and mechanical properties, has received considerable attention of researchers in the past several decades. The final properties of the blends are determined by the miscibility of the polymers, which is greatly favoured by formation of intermolecular hydrogen bonds between the component polymers (Basavaraju et al. [2]; Zhang et al. [45]; and Zheng et al. [46]). The modification of chitosan by means of blending with other polymers may be a convenient and effective method to improve physical properties for practical utilization to be applied as sorbents, biomaterials, and drug carriers (Rogovinaa et al. [26]; Twu et al. [34]; and Wub et al. [39]). The blends of cellulose and chitosan present special interest; while fibres and films made from pure chitosan have only moderate mechanical properties; a chitosan-cellulose blend can have, simultaneously, the chemical functionality of chitosan and the good mechanical properties of cellulose (Urreaga & Orden [35]).

In the present study, the new blended films of O-CMCS and viscose have been prepared and characterized. Antibacterial tests are studied to examine the possibility of O-CMCS /viscose blended films to be used as a biomaterial for wound dressing.

2. Experimental Works

2.1. Material

Chitosan (CS) (Aldrich, viscosity 1860cps, degree of deacetylation 79.0%). All other chemicals and reagents were of analytical grade, and were used without further purification. Staphylococcus aureus (S. aureus), Bacillus subtilis (B. subtilis,), Escherichia coli (E. coli), and Proteus used for the antibacterial assay were provided by the Faculty of Girls for Arts, Science and Education, Ain Shams University, Cairo, Egypt.

2.2. Methods

2.2.1. Carboxymethylation of chitosan

The carboxymethylation of chitosan was performed based on (Joshi & Sinha [16] and Zhu et al. [48]), with some modifications. Briefly chitosan (5g), sodium hydroxide (10-70%), isopropanol (80ml), and water (20ml) were added into a three necked flask (250ml) to swell and alkaline at room temperature for one hour. The monochloroacetic acid (1-5M) was dissolved in isopropanol (20ml), and added into the reaction mixture drop wise for 30 minutes and reacted for proper time (0.5-12 hrs) at temperature (30-90°C), then stopped by adding 80% ethyl alcohol. The solid was filtered and rinsed in 70-90% ethyl alcohol to desalt and dewater and dried at room temperature, followed by estimation of nitrogen and carboxylic contents. O-O-CMCS is water soluble at a wide range of pH. Factors affecting the carboxymethylation reaction are studied in details.

2.2.2. Preparation of cellulose xanthate (viscose)

Cellulose xanthate is prepared according to the reported method (Liang et al. [20] and Kaller et al. [17]), with some modifications. About 10g cellulose pulp was dispersed in with 200ml 20% NaOH solution at 25°C for 1hr in order to convert cellulose into alkali cellulose that might be exposed to air for 44 hrs to be aged. Alkali cellulose is placed in a sealed bottle with 50g carbon disulphide for 18 hrs with occasional shaking at 25°C to form cellulose xanthate. The obtained orange cellulose xanthate jelly was dissolved in 5% (W/V) NaOH aqueous solution and was allowed to ripen at 25°C for 12 hrs to obtain a dense cellulose xanthate solution (i.e., viscose). The content of cellulose in it was 5% (W/W).

2.2.3. Forming of O-CMCS / viscose blended film

To prepare O-CMCS blended with viscose in the form of a film, viscose and O-CMCS are dissolved in aqueous 5% NaOH solution to yield 9wt% solution, respectively. Viscose and O-CMCS were blended in different ratios (9/1, 8/2, 7/3, 6/4, 5/5, 4/6, 3/7, 2/8, and 1/9 w/w) to yield transparent films. The films prepared are left overnight and then soaked in a coagulation bath consisting of aqueous 10% H_2SO_4, 20% Na_2SO_4, 1% $ZnSO_4$, 1% glucose, and 68% ethanol. After washing with distilled water, the films are soaked in a 2% Na_2SO_3 solution for 20 minutes, and finally washed with distilled water and dried at 50°C in an air oven.

2.3. Analytical methods

Nitrogen content was determined by using micro-Kehjeldal procedure (Vogel [37]), carboxyl content of O-CMCS was determined according to a reported method (Daul et al. [8]). The DS values, which indicate the progress of carboxymethylation reaction, were measured by analyzing the carboxymethyl group on anhydroglucosamine unit (AGU) of starch by back titration (Stojanovic et al. [31]). Tensile properties for the blended films with thickness $55\mu m$ were measured according to ASTM-D6693-01 standard method (LR 5K, LLOYD, England), Fourier transform infrared (FT-IR) spectra of the samples were recorded by using an FT-IR spectrophotometer (Nexus 670, Nicolet, USA) in the region of $4000\text{-}400 cm^{-1}$ with spectra resolution of $4 cm^{-1}$, and the thermal analysis experiments were conducted by using simultaneous thermal gravimetric analyzer (Perkin Elmer thermo-gravimetric analyzer, TGA7, the heating rate is 10°C/min., USA). The surface morphology of blended film were studied by scanning electron microscope (SEM) examination by mounting the samples on stub with double stick adhesive tape and coated with gold in a S150A sputter coater unit (Edwards, UK). The gold film thickness was 150A°. The samples were then viewed in a JEOL JXA-840 electron probe microanalyser, Japan. The membranes for SEM were frozen in liquid nitrogen, immediately snapped, then freeze-dried under vacuum at – 60°C and cover by gold vapours.

2.4. Evaluation of antibacterial activity in vitro

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of CS and O-CMCS were determined by broth tube dilution method (Mazzola et al. [23]), the inhibition zone of O-CMCS

/viscose blended films were determined by disk diffusion method on an agar plate (Miller et al. [24]), and the reduction percentage of these blended films were determined by colony counting method (Chung & Chen [6]).

In the antibacterial assay, all data were the means from at least three parallel experiments that the discrepancies among them were less than 5%.

3. Results and Discussion

3.1. Carboxymethylation of chitosan

A series of carboxymethylation reactions were performed in this work in order to study the effect of four reaction parameters, namely, the amounts of MCAA (1-5 molar solution), NaOH (10-70% g/g), the reaction time (0.5-12 hrs), and reaction temperature (30-90ºC) on the degree of substitution (DS) and the water-solubility at different pH of O-CMCS. Different ranges of each parameter were chosen based on previous studies (Zamani et al. [44]; Chen & Park [40]; Chen et al. [5]; and Sabaa et al. [27]) and these tests listed in Table 1. Other factors that affect the progress of carboxymethylation reaction, for example, the ratio of $H_2O/2\text{-}PrOH/$ chitosan was set around optimal a value according to the previous studies (Chen & Park [40] and Kurite [19]), which was 2:10:1. The FT-IR spectra of all the O-CMCSs prepared in this study were similar, and an example is shown in Figure 1. In these spectra, the wide band at 3420cm^{-1} corresponds to the axial stretching of the O–H and N–H bonds (De-Abreu & Camara-Filho [10]). The peaks at 2927cm^{-1} and 1639cm^{-1} are attributed to the axial stretching of the C–H bonds and the symmetric stretching vibration of C=O in the –COOH groups, respectively (Chen & Park [40] and Ge & Luo [11]). The latter peak, together with the peak at 1420cm^{-1}, which arose from the asymmetric stretching vibration of the $-\ COO^{-}$ group, confirm the substitution of carboxymethyl groups onto the chitosan chain (De-Abreu & Camara-Filho

[10] and Ge & Luo [11]). Two bands at 1528 and 1513cm^{-1} assigned to NH_3^+, indicate that the carboxymethylation occurred at OH positions (Wang & Wang [38] and Chen & Park [40]). The peaks at 1413 and 1377cm^{-1} are related to the symmetric angular deformation of C–H bonds and C–N stretching vibrations (amide III band), respectively (De-Abreu & Camara-Filho [10] and Ge & Luo [11]). The peak at 1377cm^{-1} did not increase significantly in the spectra of the O-CMCSs, compared to the chitosan spectrum, which indicates that a significant amount of N-carboxymethylation did not take place (Ge & Luo [11]). The stretching vibration of C–O in the CH_2COOH group gives rise to the peak at 1207cm^{-1}. Peaks located in the range of 1175-878cm^{-1} are the result of vibrations of C–O and C–O–C and some other bonds that comprise the polysaccharide chain (De-Abreu & Camara-Filho [10]).

3.1.1. Optimization of the reaction parameters

The carboxymethylation of chitosan proceeds by a two step consecutive reaction and is accompanied by an undesired side reaction. In the main reaction, the sodium hydroxide reacts first with the hydroxyl groups of chitosan to give alkali chitosan. The carboxymethyl groups are then formed in an SN_2 reaction between the alkali chitosan and monochloroacetic acid (MCAA). The main reaction is given by

$$\text{Chit-OH} + \text{NaOH} \longrightarrow \text{Chit-ONa} + H_2O. \tag{1}$$

$$\text{Chit-ONa} + ClCH_2COOH \longrightarrow \text{Chit-OCH}_2\text{COOH} + NaCl. \tag{2}$$

The side reaction takes place and results in the formation of sodium glycolate from MCAA and sodium hydroxide.

$$NaOH + ClCH_2COOH \longrightarrow HOCH_2COONa + NaCl. \tag{3}$$

The optimization of the process of carboxymethylation was performed by varying the process parameters, such as concentration of NaOH and MCAA, temperature, and duration of the reaction. Each parameter was varied keeping the others constant as shown in Table 1.

Table 1. Effect of NaOH, monochloroacetic acid concentration, reaction period, and reaction temperature on DS of reacted carboxylic groups

Factor studied		Nitrogen content N%	Carboxyl content COOH%	DS
[NaOH]%	10	4.40	3.11	0.15
	20	4.15	6.01	0.29
	30	4.00	9.53	0.46
	40	3.97	11.80	0.57
	50	3.90	13.06	0.63
	60	3.94	12.23	0.59
	70	4.10	5.59	0.27
[MCAA] M (Molarity)	1	4.12	6.01	0.29
	2	3.86	12.23	0.59
	2.5	3.90	13.06	0.63
	3	3.60	14.10	0.68
	4	3.45	17.21	0.83
	5	2.90	22.39	1.08
Reaction Period (hrs)	0.5	4.20	6.43	0.31
	1	3.90	9.54	0.46
	2	3.78	12.23	0.59
	3	3.90	13.06	0.63
	6	3.00	16.79	0.81
	9	2.90	22.81	1.10
	12	2.70	25.09	1.21
Reaction Temperature (°C)	30	5.60	1.03	0.05
	40	4.40	3.11	0.15
	50	4.05	9.33	0.45
	60	3.90	13.06	0.63
	70	4.01	12.23	0.59
	80	4.09	11.80	0.57
	90	4.15	9.53	0.46

Experimental conditions used: [chitosan], 5gm; [NaOH], 50%; [MCAA], 2.5M; reaction temperature, 60°C; reaction time: 3hrs; and material to liquor ratio (M: LR), 1:20.

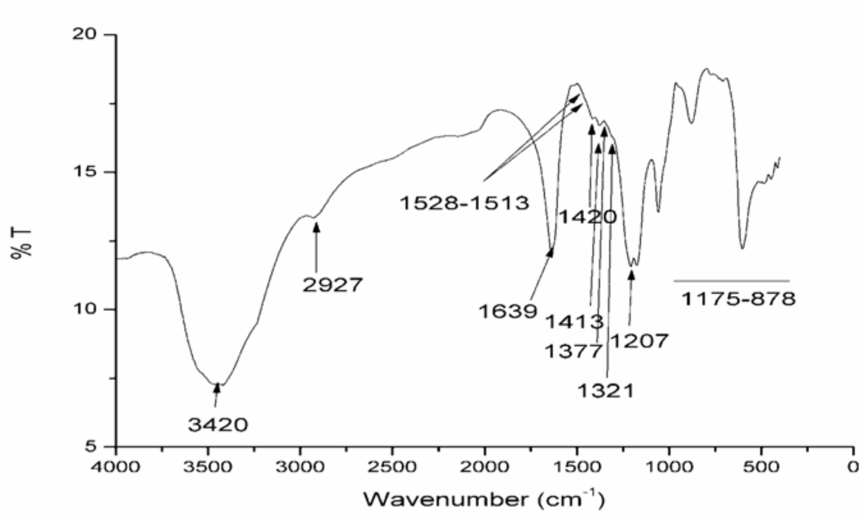


Figure 1. FTIR spectrum of the CMCS prepared by reaction of 5gm chitosan with 2.5M MCAA in the presence of 50% NaOH within 3 hrs at 60ºC.

Effect of sodium hydroxide concentration

The effect of variation of sodium hydroxide concentration from 10% to 70% (w/v) at 60ºC on DS was studied and the results are shown in Table 1. It was observed that, as sodium hydroxide concentration increases up to 50%, the DS also increases and then level off. The increase in DS with increase in NaOH concentration up to 50% suggests that the carboxymethylation reaction shown by Equation (2) prevails over its competitive reaction shown by Equation (3). Above 50% concentration of NaOH, the glycolate formation increases (Goyal et al. [12]; Gupta et al. [13]; Chen et al. [5]; Sharma et al. [28]; Stojanovic et al. [30]; and Verraest et al. [36]), and consequently, a lower value of the DS of the O-CMCS sample was obtained. Therefore, the 50% concentration of NaOH constitutes the optimum concentration for carboxymethylation of chitosan.

Effect of monochloroacetic acid concentration

The effect of MCAA concentration on the extent of carboxymethylation reaction of chitosan was studied by using the optimum concentration of NaOH (50%). Table 1 shows the effect of monochloroacetic acid concentration on DS of carboxyl groups in carboxymethylation of chitosan. It is clear that the DS of carboxyl groups increase significantly as the concentration of MCAA increases up to 2.5M, and still increase with increasing the concentration of monochloroacetic acid to 5M, but this increase in substrate concentration is useless for the carboxymethylation reaction due to the formation of glycolate in Equation (3), which seems to be more pronounced at high concentrations of MCAA, and there are some undesirable gels formed in the reaction medium and due to the aggregation of highly acetylated chain segmented or to amide formation subsequent of thermal drying (Chen & Park [40]). Thus, 2.5mol/L $ClCH_2COOH$ is the optimum condition for carboxymethylation reaction of chitosan.

Effect of reaction time

Table 1 illustrates the effect of carboxymethylation reaction time on DS of carboxyl groups in O-CMCS. It is clear that the extent of carboxymethylation of chitosan increases by prolonging the duration of reaction. This is rather direct consequence of the favourable effect of time on swellability of chitosan as well as diffusion and adsorption of reactants with the ultimate effect of including better contacts between etherifying agent and chitosan thereby promoting ether formation through improved carboxymethylation process (Goyal et al. [12]; Gupta et al. [13]; Sharma et al. [28]; and Stojanovic et al. [30]). But after 3 hours, there are some polymer degradation of O-CMCS and the formation of glycolate, which seems to be more pronounced at higher duration and there are some undesirable gels formed in the reaction medium. Thus, 3 hours reaction time is the optimum condition for carboxymethylation reaction of chitosan.

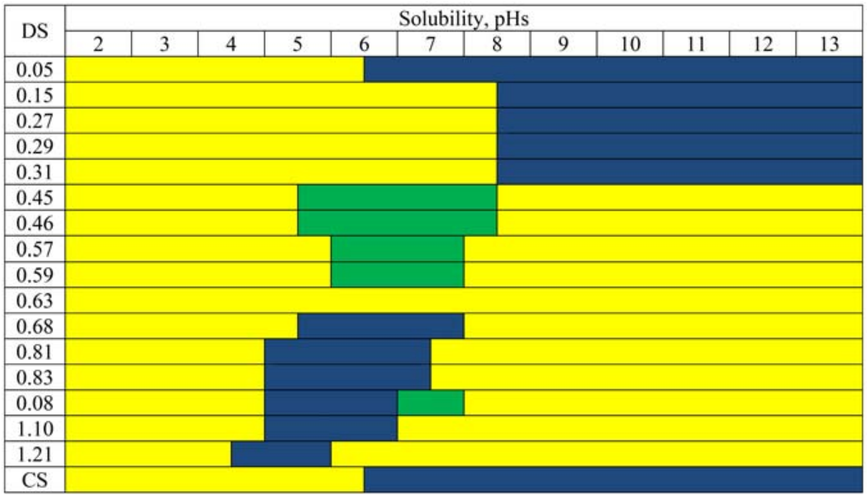
Effect of reaction temperature

Carboxymethylation of chitosan was performed at different temperatures (i.e., 30-90ºC). The dependence of DS on reaction temperature is shown in Table 1. It is observed that DS increases from 0.05 to 0.63 prominently as the reaction temperature increases from 30 to 60°C and decreases thereafter. It is due to the favourable effect of temperature on the swellability of chitosan as well as the diffusion and adsorption of reactants with the ultimate effect of inducing better contacts between the etherifying agents and chitosan. The value of DS decreases at 90ºC, due to the higher glycolate formation Equation (3). (Goyal et al. [12]; Gupta et al. [13]; and Sharma et al. [28]) prevailing over the carboxymethylation Equation (2). Thus, 60ºC reaction temperature is the optimum condition for carboxymethylation reaction of chitosan.

3.1.2. Solubility of O-CMCS in water at different pHs

The water solubility of O-CMCS was observed in by measuring the turbidity of aqueous solutions of O-CMCSs at different pH according to Sashiwa and Shigemasa [9]. As shown in Table 2, the initial reactant chitosan was soluble in water at a slightly acidic pH ($pH < 6.5$) and insoluble over neutral and alkaline pH. The majority of O-CMCSs prepared in this work exhibited a limited insoluble region around neutral pH, while they were soluble in acidic and alkaline pH. The DS of carboxymethyl groups had higher impact on the water solubility of O-CMCS. At lower DS values (0.005-0.46), the O-CMCSs were either insoluble or partially soluble over the entire pH range (2-13). At higher DS values (0.59-0.68), however, O-CMCSs with improved water solubility were obtained. At DS higher than 0.68, an insoluble region moving to lower pH close to $pH = 7$, due to the aggregation of highly acetylated chain segmented or to amide formation subsequent of thermal drying (Chen & Park [40]). In summary, the widest soluble range of pHs was obtained for $DS = 0.63$.

Table 2. Solubility of chitosan and CMCS in water at different pHs and relation to DS



DS	Solubility, pHs											
	2	3	4	5	6	7	8	9	10	11	12	13
0.05	🟨	🟨	🟨	🟨	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦
0.15	🟨	🟨	🟨	🟨	🟨	🟨	🟦	🟦	🟦	🟦	🟦	🟦
0.27	🟨	🟨	🟨	🟨	🟨	🟨	🟦	🟦	🟦	🟦	🟦	🟦
0.29	🟨	🟨	🟨	🟨	🟨	🟨	🟦	🟦	🟦	🟦	🟦	🟦
0.31	🟨	🟨	🟨	🟨	🟨	🟨	🟦	🟦	🟦	🟦	🟦	🟦
0.45	🟨	🟨	🟨	🟩	🟩	🟩	🟨	🟨	🟨	🟨	🟨	🟨
0.46	🟨	🟨	🟨	🟩	🟩	🟩	🟨	🟨	🟨	🟨	🟨	🟨
0.57	🟨	🟨	🟨	🟨	🟩	🟩	🟨	🟨	🟨	🟨	🟨	🟨
0.59	🟨	🟨	🟨	🟨	🟩	🟩	🟨	🟨	🟨	🟨	🟨	🟨
0.63	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
0.68	🟨	🟨	🟨	🟦	🟦	🟦	🟨	🟨	🟨	🟨	🟨	🟨
0.81	🟨	🟨	🟨	🟦	🟦	🟨	🟨	🟨	🟨	🟨	🟨	🟨
0.83	🟨	🟨	🟨	🟦	🟦	🟨	🟨	🟨	🟨	🟨	🟨	🟨
0.08	🟨	🟨	🟨	🟦	🟦	🟩	🟨	🟨	🟨	🟨	🟨	🟨
1.10	🟨	🟨	🟨	🟦	🟦	🟨	🟨	🟨	🟨	🟨	🟨	🟨
1.21	🟨	🟨	🟦	🟦	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
CS	🟨	🟨	🟨	🟨	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦

*Solid sample (10mg) was dispersed in H_2O (50ml) on the base of method of Sashiwa and Shigemasa [9].

*Soluble (yellow colour), partially soluble (green colour), and insoluble (blue colour).

3.2. Characterization of O-CMCS /viscose blended films

The films prepared based on blends of O-CMCS and viscose were transparent and brittle in a dry state. An immersion of the films in deionized water for over 24 hrs leads to their disintegration or partial dissolution. The films were examined by nitrogen content, tensile properties, Fourier-transform infrared spectroscopy (FTIR), thermal gravimetric analysis (TGA), and scanning electron microscopy (SEM).

3.2.1. Nitrogen content and mechanical properties

Table 3 illustrates that as O-CMCS percent increase in the blended film, both %N content and elongation-at-break (E) increases in a regular trend, although the tensile strength (TS) decreases in a regular trend, which indicate there is high homogeneity in the distribution of the two components within the blended film. The increasing TS values of the composite films, with the viscose to O-CMCS ratio increasing from 0% to

100%, are attributable to the formation of inter-molecular hydrogen bonds between NH_2 and COOH of the O-CMCS backbone and OH of the viscose. The amino groups (NH_2) of the O-CMCS were protonated to NH_3^+ by COOH in the solvent, , resulting in the OH groups being exposed to readily form hydrogen bonds with NH_2 and COOH of the O-CMCS. The number of OH groups increased with increasing viscose ratio in the film forming solution. The decrease in E with increasing viscose ratio may occur because there are also at the same time intra-molecular hydrogen bonds between HN_2 and COOH in, resulting in a phase separation between the two main components (Xu et al. [42] and Wu et al. [39]).

Table 3. Percent nitrogen content, tensile strength, and elongation yield% of viscose / CMCS blended films

	Film composition	Nitrogen content N%	Tensile strength MPa (N/m^2)	Elongation yield %
Viscose: CMCS	100 : 0	0.00	5.89	5.59
	90 : 10	0.15	5.12	5.48
	80 : 20	0.31	4.53	5.36
	70 : 30	0.42	4.39	5.14
	60 : 40	0.62	4.13	5.00
	50 : 50	0.81	4.08	4.96
	40 : 60	0.82	3.92	4.72
	30 : 70	1.51	3.78	4.69
	20 : 80	1.81	3.41	4.53
	10 : 90	2.22	2.89	3.67
	0 : 100	3.90	1.99	5.67

3.2.2. FTIR analysis

FTIR spectroscopy was used to examine the interactions between O-CMCS and viscose. The infrared spectra of O-CMCS, viscose, and O-CMCS/viscose blended film are presented in Figure 2 and Table 4. The O-CMCS spectrum was similar to previous reports (Bidgoli et al. [3] and Chen & Park [40]) as mentioned in the carboxymethylation, also the viscose spectrum in Figure 2 and Table 4 was similar to previous reports (Yin et al. [43] and Cai & Kim [50]). When two or more substances are mixed, physical blends versus chemical interactions are reflected by changes in characteristic spectra peaks (Xu et al. [42]). In the typical spectrum of O-CMCS/viscose blended films, the −C=O vibration for COOH group peak of O-CMCS shifted from 1639 to 1636cm^{-1} with the addition of viscose and disappeared in the viscose film. This result indicated that interactions were present between the OH groups of viscose and the COOH and NH_2 groups of O-CMCS, and the absorption band at 2922cm^{-1} corresponding to CH-stretching decreases in intensity by increasing the O-CMCS ratio from 20% to 40% to 60% and almost overlapped at ratio of 20:80 viscose: O-CMCS, which might indicate also good homogeneity in the distribution of the two components within the blended film. In general, it could be concluded that comparison of spectra of O-CMCS and blended films with viscose show the OH-stretching vibration band of the blended films shifted to a lower wave number and broaden. Moreover, the NH-stretching band of chitosan derivatives disappeared in the blended films. These results indicate that strong intermolecular hydrogen bonding interaction takes places between viscose and O-CMCS in the blended films, leading to a good homogeneity of the blends.

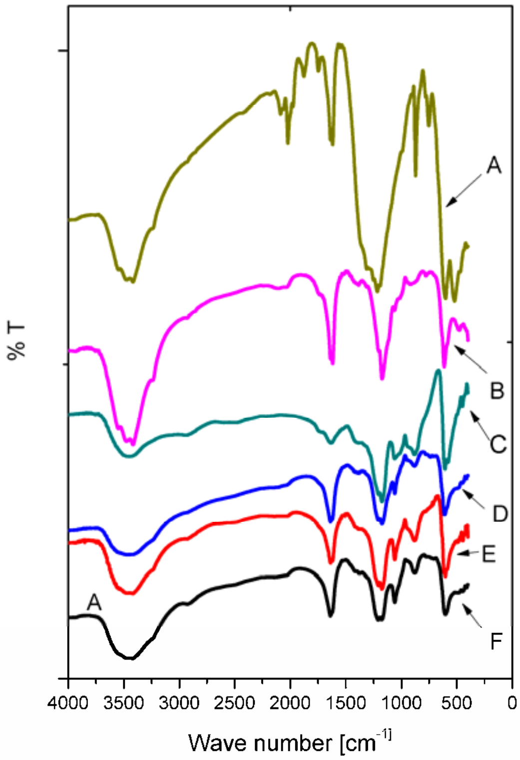


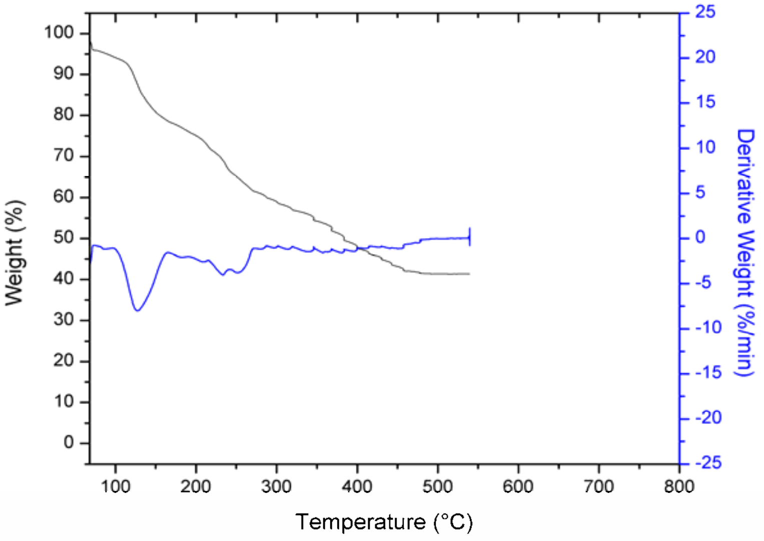
Figure 2. FTIR spectra of viscose (a), viscose: CMCS blended films 80:20 (b); 60:40 (c); 40:60 (d); 20:80 (e); and CMCS film (f).

Table 4. FTIR absorption bands in viscose/CMCS blends

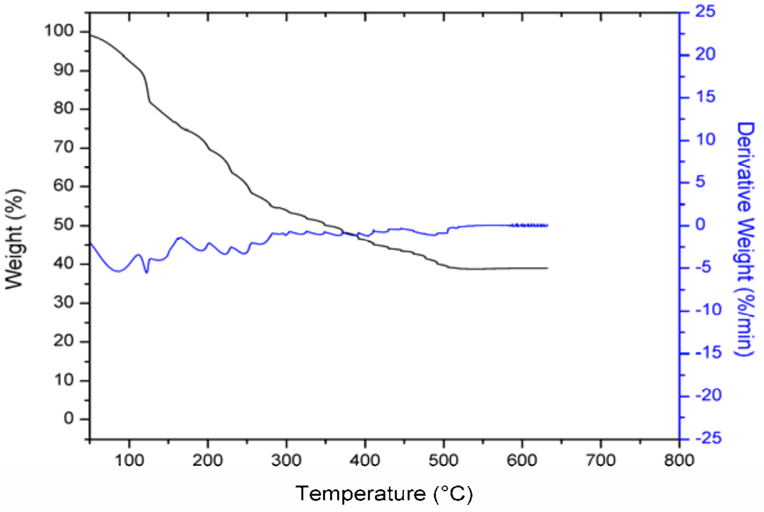
Blend Viscose/CMCS, wt%						
0:100, cm^{-1}	20:80, cm^{-1}	40:60, cm^{-1}	60:40, cm^{-1}	80:20, cm^{-1}	100:0, cm^{-1}	Assignment
3475 3416	3475 3416	3470	3475	3479 3414	3420	– OH and – NH stretching
–	2929	2922	2925	2925	2922	– CH stretching
–	1638 1618	1633	1638	1637 1619	1639	–C = O vibration for COOH group
1215	1200	1208	1206	1205	1207	Anti-symmetric stretching of C–O–C bridge
1192	1172	1171	1173	1174	1175	
–	1061	1064	1059	1059	1058	Skeletal vibrations involving the C = O stretching

3.2.3. Thermogravimetric analysis (TGA)

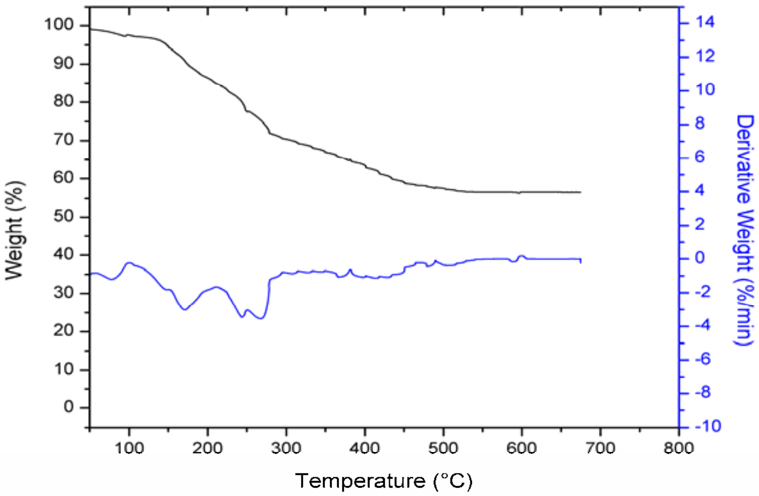
The thermal stability and thermal decomposition of viscose, O-CMCS and viscose/O-CMCS blends were investigated by using TGA and are given in Figure 3. It shows that for O-CMCS and viscose and based on thermal nature of these polymers (decarboxylation, evolution of CO_2, and dehydration) by increasing temperature. The results show that thermogravimetric analysis is not suitable to differentiate between these two polymers.



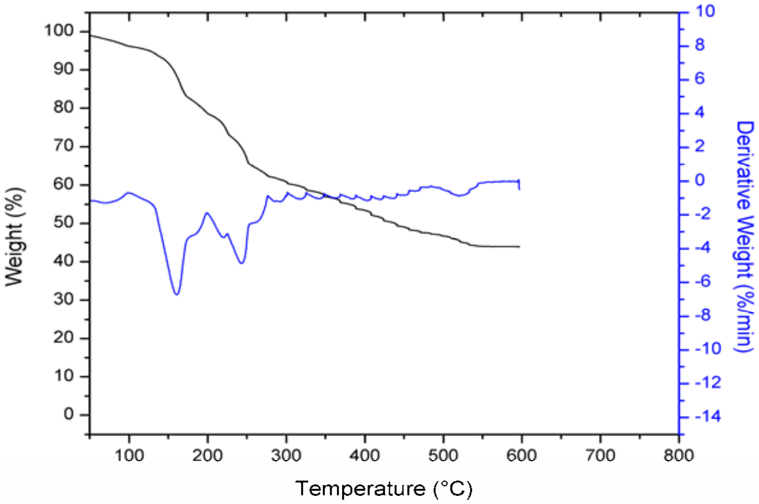
(a) CMCS/Viscose (00/100)



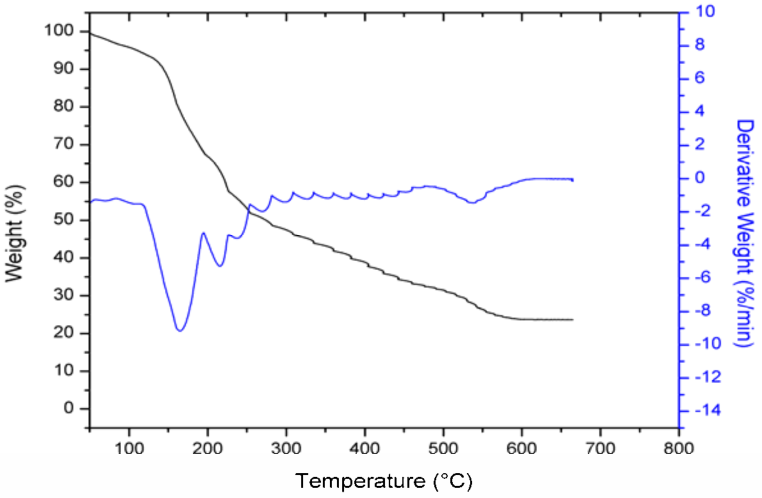
(b) CMCS/Viscose (20/80)



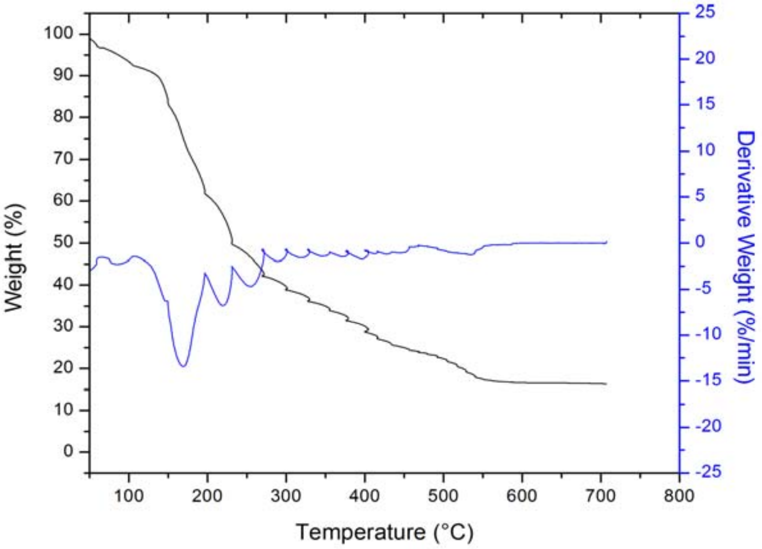
(c) CMCS/Viscose (40/60)



(d) CMCS/Viscose (60/40)



(e) CMCS/Viscose (80/20)

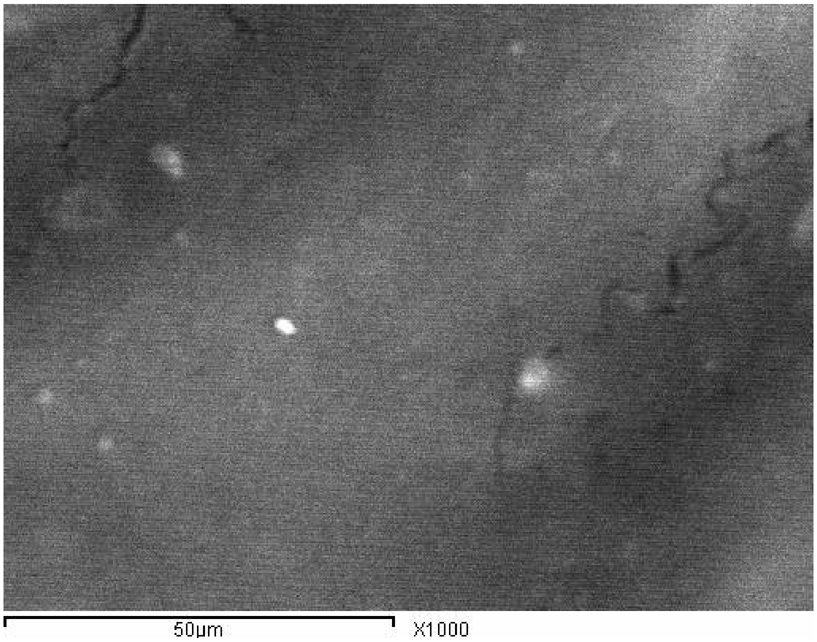


(f) CMCS/Viscose (100/00)

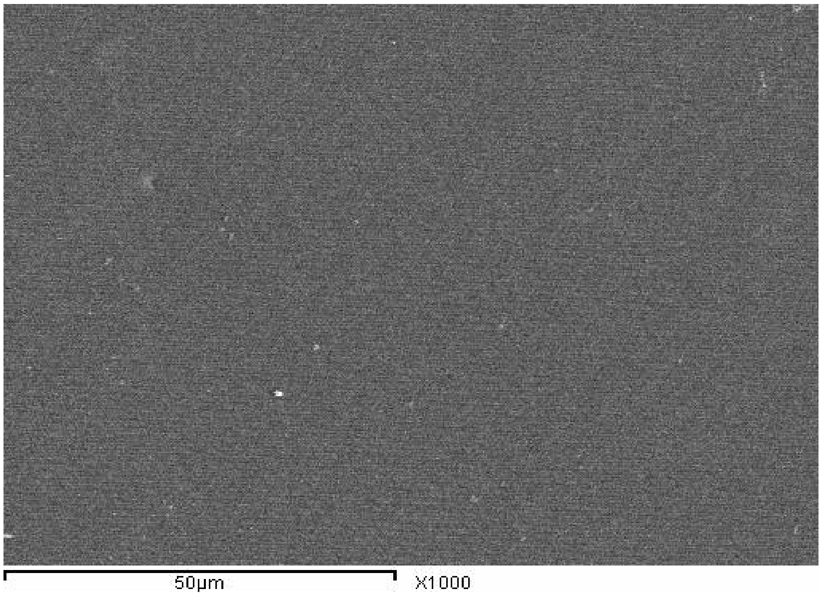
Figure 3. TGA for CMCS/viscose blended films.

3.2.4. Scanning electron microscopy (SEM)

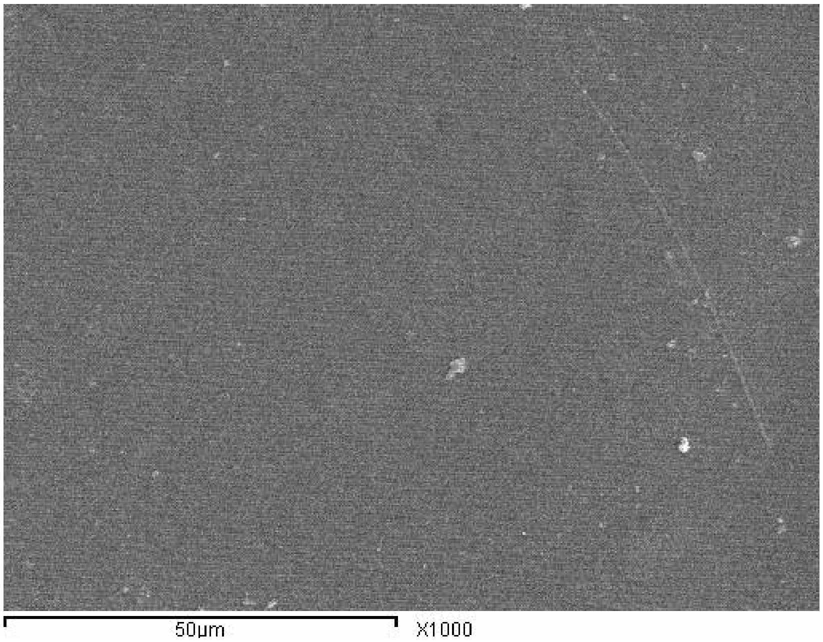
The morphology of the surface of the blended films was studied by scanning electron microscopy. SEM microphotographs of the surface of O-CMCS, viscose (control), and blend films were shown in Figure 4. It can be seen from Figure 4 ((a)-(f)) that the surface morphology of O-CMCS, viscose and their blended films are rather smooth, compact except for some small holes created by air dissolved in the solvent and shows a homogeneous structure in the surface of the blended films. The gold layer is tightly and uniformly connected with the viscose /O-CMCS blended films. In conclusion, these blends are mixed well in the films.



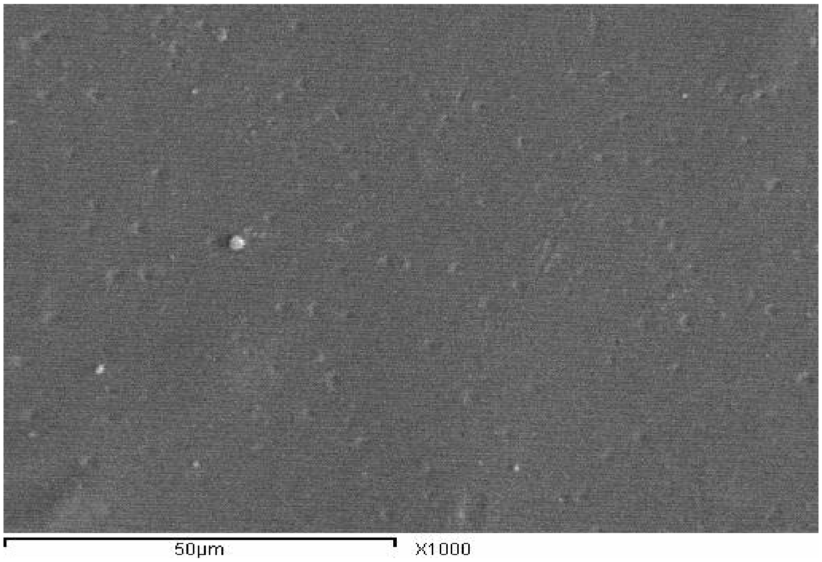
(a) CMCS: viscose; 100:00



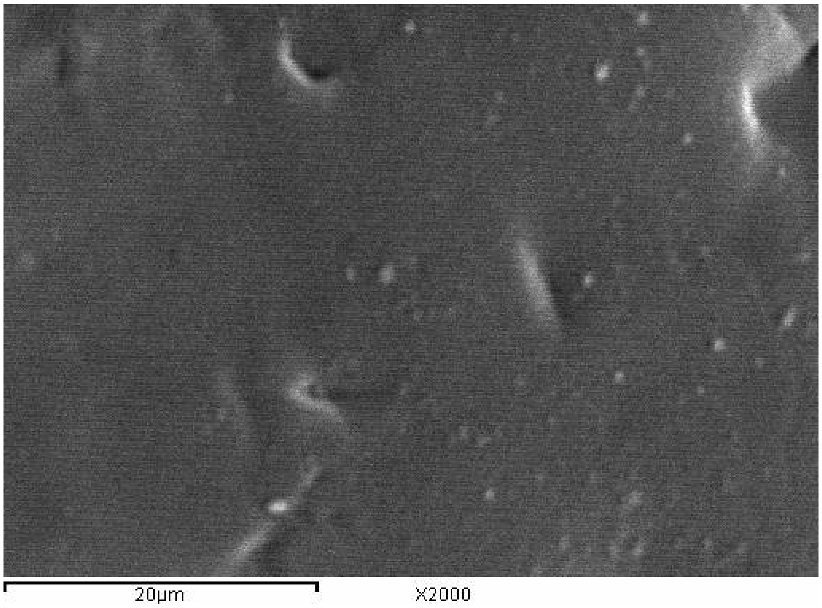
(b) CMCS: viscose; 80:20



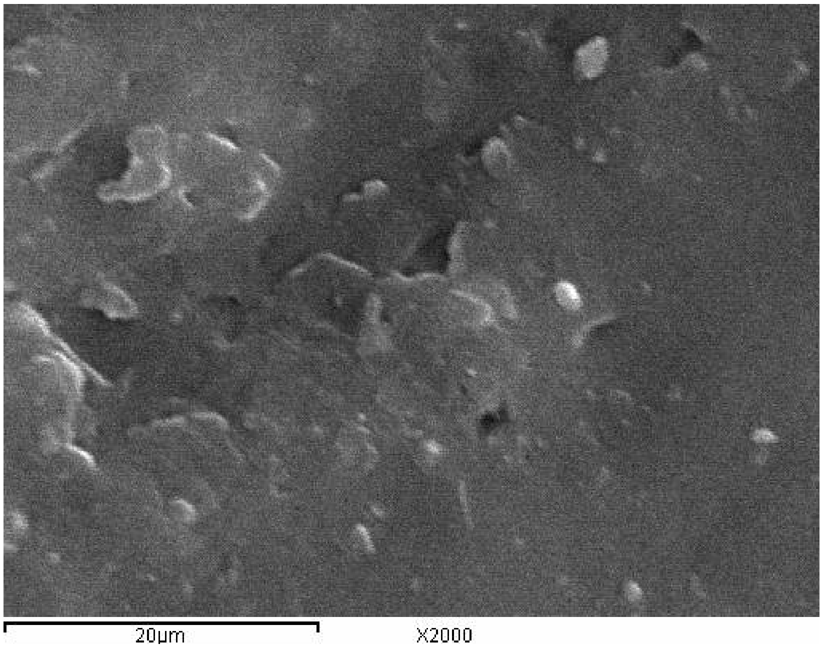
(c) CMCS: viscose; 60:40



(d) CMCS: viscose; 40:60



(e) CMCS: viscose; 20:80:



(f) CMCS: viscose; 00:100

Figure 4. SEM images of CMCS/viscose blended films.

3.3. Antibacterial properties

3.3.1. MIC and MBC for CS and O-CMCS

Table 5 shows the MIC and MBC of the CS and O-CMCS, according to the two-fold broth tube dilution method, on *S. aureus* and *E. coli*. As shown in Table 5, the MIC and MBC values slightly differed with the tested organism, where CS and O-CMCS showed stronger bactericidal effects for Gram-positive bacteria (*S. aureus*) than for Gram-negative bacteria (*E. coli*) as observed by several workers (Chung et al. [7]; Sun et al. [33]; and Xu et al. [41]). This may be due to their different cell walls. *S. aureus*, as a Gram-positive bacterium, its cell wall is fully composed of peptide polyglycogen. The peptidoglycan layer is composed of networks with plenty of pores, which allow foreign molecules to come into the cell without difficulty. But *E. coli*, as a Gram-negative bacterium, the cell wall of which is made up of a thin membrane of peptide polyglycogen and an outer membrane constituted of lipopolysaccharide, lipoprotein, and phospholipids. Because of the bilayer structure, the outer membrane is a potential barrier against foreign molecules (Kong et al. [18] and Sun et al. [33]).

MIC and MBC values reported in Table 5 shown that O-CMCS had higher antibacterial activity compared with CS, due to the antibacterial activity of CS and its derivatives based on the damaging interaction of polycation, due to a large amount of NH_3^+ in the solution, with negatively charged surface of bacteria resulting in loss of membrane permeability, cell leakage and cell death (Papineau et al. [25]; Sudharshan et al. [32]; and Zheng et al. [47]) for O-CMCS the presence of $-CH_2COOH$ groups on C-6 promote the polycation property (number of promoting NH_3^+) of O-CMCS than that of CS itself.

Table 5. MIC and MBC values found for chitosan and carboxymethylchitosan

	S. aureus		*E. coli*	
	MIC, ppm	MBC, ppm	MIC, ppm	MBC, ppm
Chitosan	78	156	625	2500
Carboxymethylchitosan	39	78	213.5	1250

3.3.2. Disk diffusion and bacterial count methods for viscose/O-CMCS polymer films

Table 6 shows the antibacterial properties of viscose, O-CMCS, and viscose/O-CMCS blended films measured by both inhibition zones and percent in bacterial growth against Gram-positive and Gram-negative bacteria. It was noted that the inhibition zone and numbers of colony expressed in mm and reduction%, respectively, of all test bacteria formed on the viscose /O-CMCS blended films increase with the increase of O-CMCS concentration in the film, and also as we mentioned before viscose /O-CMCS films had stronger bactericidal effects for Gram-positive bacteria than for Gram-negative bacteria. These results indicate a good potential of the developed viscose /O-CMCS blended films for being used as wound dressing due to their capability to bind the negatively charged bacteria to the positively charged amino groups of the chitosan polymer by reducing the primary wound contamination and due to their protection ability of the wound from secondary bacterial infection.

Table 6. Inhibition zones found and percent reduction in bacterial growth for viscose/CMCS blended polymer discs of different polymer ratios and tested against G +ve and G –ve bacteria

Polymer ratio Viscose: CMCS	Diameter of Clear Zone (mm)				Reduction %	
	Gram Positive		Gram Negative			
	S. aureus	*B. subtilis*	*E. coli*	*Proteus*	*S. aureus*	*E. coli*
100 : 0	00	00	00	00	0	0
90 : 10	00	00	00	00	15.78	4.34
80 : 20	12	00	00	00	28.94	6.95
70 : 30	12	14	00	00	92.28	16.52
60 : 40	14	15	00	00	92.45	17.39
50 : 50	15	15	00	00	93.15	24.23
40 : 60	15	16	00	00	94.03	40.00
30 : 70	15	15	12	12	97.42	55.94
20 : 80	15	15	12	12	97.43	60.86
10 : 90	17	15	12	12	97.45	64.34
0 : 100	18	16	12	13	99.59	74.20

4. Conclusion

- Carboxymethylation of chitosan was carried out with monochloroacetic acid in the presence of sodium hydroxide under different reaction conditions. Variables studied were concentration of sodium hydroxide, concentration of monochloroacetic acid, reaction time, and reaction temperature, to obtain the optimum conditions for carboxymethylation.

- The optimum reaction conditions for carboxymethylation of chitosan are: [monochloroacetic acid] = 2.5M; [NaOH] = 50%; reaction temperature = 60°C, and reaction period = 3 hrs, to give the widest soluble range of pHs was obtained for DS = 0.63.

- CS and O-CMCS showed stronger bactericidal effects for Gram-positive bacteria (*S. aureus*) than for Gram-negative bacteria (*E. coli*) and O-CMCS had higher antibacterial activity compared with CS.

- O-CMCS/viscose blended were prepared successfully by mixing O-CMCS and viscose in water as co-solvent, and regenerated in acidic coagulation bath as mentioned in experimental part. The mechanical properties of O-CMCS film was improved after incorporation of viscose into the blended films and the antibacterial activity of the blended films increased by increasing the O-CMCS percent in the blended films.

- The miscibility of O-CMCS/viscose blended films has been assessed by SEM.

- Viscose/O-CMCS blended films developed to be used as wound dressing due to their capability to bind the negatively charged bacteria to the positively charged amino groups of the chitosan polymer by reducing the primary wound contamination and due to their protection ability of the wound from secondary bacterial infection.

Acknowledgement

The authors gratefully acknowledge chemist Ahmed Abdel-Tawab Bakr (Hema-plast Company, Egypt) for cooperation in performing the mechanical measurements and related measurements.

References

[1] D. Angewandte, Pervaporation separation of water-ethanol through modified chitosan membranes, Makromolekulare Chemie 192 (1991), 169-181.

[2] K. C. Basavaraju, T. Damappa and S. K. Rai, Preparation of chitosan and its miscibility studies with gelatin using viscosity, ultrasonic and refractive index, Carbohydrate Polymers 66 (2006), 357-362.

[3] H. Bidgoli, A. Zamani and M. J. Taherzadeh, Effect of carboxymethylation conditions on the water-binding capacity of chitosan-based superabsorbents, Carbohydrate Research 345 (2010), 2683-2689.

[4] Cassia occidentalis seed gum, Journal of Applied Polymer Science 94, 1606-1611.

[5] L. Chen, Y. Du and X. Zeng, Relationships between the molecular structure and moisture-absorption and moisture-retention abilities of carboxymethyl chitosan II, Effect of degree of deacetylation and carboxymethylation, Carbohydrate Research 338 (2003), 333-340.

[6] Y. C. Chung and C. Y. Chen, Antibacterial characteristics and activity of acid-soluble chitosan, Bioresource Technology 99 (2008), 2806-2814.

[7] Y. V. Chung, Y. P. Su, C. C. Chen, G. Jia, H. I. Wang, J. C. G. Wu and J. G. Lin, Relationship between antibacterial activity of chitosan and surface characteristics of cell wall, Acta Pharma. Sin. 25(7) (2004), 932-936.

[8] G. C. Daul, R. M. Reinhardt and J. D. Reid, The carboxymethylation of cotton, Textile Research Journal 23 (1953), 719-726.

[9] H. Sashiwa and Y. Shigemasa, Chemical modification of chitin and chitosan 2: Preparation and water-soluble property of N-acetylated or N-alkylated partially deacetylated chitins, Carbohydrate Polymers 39 (1999), 127-138.

[10] F. R. De Abreu and S. P. Camara-Filho, Characteristics and properties of carboxymethylchitosan, Carbohydrate Polymers 75 (2009), 214-221.

[11] H. Ge and D. Luo, Preparation of carboxymethyl chitosan in aqueous solution under microwave irradiation, Carbohydrate Research 340 (2005), 1351-1356.

[12] P. Goyal, V. Kumar and P. Sharma, Carboxymethylation of tamarind kernel powder, Carbohydrate Polymers 69 (2007), 251-255.

[13] G. Gupta, P. Sharma and P. L. Soni, Carboxymethylation of cassia occidentalis seed gum, Journal of Applied Polymer Science 94 (2004), 1606-1611.

[14] I. M. Helander, E. L. Nurmiaho-Lassila, R. Ahvenainen, J. Rhoades and S. Roller, Chitosan disrupts the barrier properties of the outer membrane of gram-negative bacteria, International Journal of Food Microbiology 71 (2001), 235-244.

[15] A. Isogai and R. H. Atalla, Preparation of cellulose-chitosan polymer blends, Carbohydrate Polymers 19 (1992), 25-28.

[16] J. M. Joshi and V. K. Sinha, Graft copolymerization of 2-hydroxyethylmethacrylate onto carboxymethylchitosan using CAN as an initiator, Polymer 47 (2006), 2198-2204.

[17] A. L. Kaller, Z. I. Safronova and E. M. Mogileveskii, Viscose making with double steeping, Fiber Chem. 11 (1979), 85-91.

[18] M. Kong, X. Chen, G. Chitos, C. S. Liu, C. G. Liu, X. H. Meng and L. J. Yu, Antibacterial mechanism of chitosan microspheres in a solid dispersing system against E. coli, Colloids and Surfaces B: Biointerfaces 65 (2008), 197-202.

[19] K. Kurite, Controlled functionalization of the polysaccharide chitin, Prog. Polym. Sci. 26 (2001), 1921-1971.

[20] S. Liang, X. Guo, N. Feng and Q. Tian, Application of orange peel xanthate for the absorption of Pb^{2+} from aqueous solutions, Journal of Hazardous Materials 170(1) (2009), 425-429.

[21] X. Liu, Q. Chen and H. Pan, Rheological behaviour of chitosan derivative/cellulose polyblends from N-methylmorpholine N-oxide/H_2O solution, Mater. Sci. 42 (2007), 6510-6514.

[22] G. Ma, D. Yang, Y. Zhou, M. Xiao, J. F. Kennedy and J. Nie, Preparation and characterization of water soluble N-alkylated chitosan, Carbohydrate Polymers 74 (2008), 121-126.

[23] P. G. Mazzola, A. F. Jozala, L. C. L. Novaes, P. Moriel and T. C. V. Penna, Minimal inhibitory concentration (MIC) determination of disinfectant and/or sterilizing agents, Brazilian Journal of Pharmaceutical Sciences 45(2) (2009), 241-248.

[24] R. A. Miller, R. D. Walker, A. Baya, K. Clemens, M. Coles, J. P. Hawke, B. E. Henricson, H. M. Hsu, J. J. Mathers, J. L. Oaks, M. Papapetropoulou and R. Reimschuessel, Antimicrobial susceptibility testing of aquatic bacteria: Quality control disk diffusion ranges for Escherichia coli ATCC 25922 and Aeromonas salmonicida subsp. Salmonicida ATCC 33658 at 22 and 28ºC, Journal of Clinical Microbiology 41(9) (2003), 4318-4323.

[25] A. M. Papineau, D. G. Hoover, D. Knorr and D. J. Farkas, Antimicrobial effect of water-soluble chitosans with high hydrostatic pressure, Food Biotechnology 5 (1991), 45-57.

[26] S. Z. Rogovinaa, T. A. Akopovab, G. A. Vikhorevac and I. N. Gorbachevac, Solid state production of cellulose-chitosan blends and their modification with the diglycidyl ether of oligo(ethylene oxide), Polymer Degradation and Stability 73 (2001), 557-560.

[27] M. W. Sabaa, N. A. Mohamed, R. R. Mohamed, N. M. Khalil and S. M. Abd El Latif, Synthesis, characterization and antimicrobial activity of poly (N-vinyl imidazole) grafted carboxymethyl chitosan, Carbohydrate Polymers 79 (2010), 998-1005; Science 89 (2010), 3216-3219.

[28] B. R. Sharma, V. Kumar, P. L. Soni and P. Sharma, Carboxymethylation of Cassia tora gum, Journal of Applied Polymer (2003).

[29] C. M. Shih, Y. T. Shieh and Y. K. Twu, Preparation and characterization of cellulose/chitosan blend films, Carbohydrate Polymers 78 (2009), 169-174.

[30] Z. Stojanovic, K. Jeremic and S. Jovanovic, Synthesis of carboxymethyl starch, Starch/Stärke 52 (2000), 413-419.

[31] Z. Stojanovic, K. Jeremic, S. Jovanovic and M. D. Lechner, A comparison of some methods for the determination of the degree of substitution of carboxymethyl starch, Starch/Starke 57 (2005), 79-83.

[32] N. R. Sudharshan, D. G. Hoover and D. Knorr, Antibacterial action of chitosan, Food Biotechnology 6 (1992), 257-272.

[33] L. Sun, Y. Du, L. Fan, X. Chen and J. Yang, Preparation, characterization and antimicrobial activity of quaternized carboxymethylchitosan and application as pulp-cap, Polymer 47 (2006), 1796-1804.

[34] Y. K. Twu, H. Huang, S. Y. Chang and S. L. Wang, Preparation and sorption activity of chitosan/cellulose blend beads, Carbohydrate Polymers 54 (2003), 425-430.

[35] J. M. Urreaga and M. U. Orden, Chemical interactions and yellowing in chitosan-treated cellulose, European Polymer Journal 42 (2006), 2606-2616.

[36] D. L. Verraest, J. A. Peters, J. G. Batelaan and H. V. Bekkum, Carboxymethylation of inulin, Carbohydrate Research 271 (1995), 101-112.

[37] A. I. Vogel, Text Book of Quantitative Chemical Analysis, 5th Revised by G. H. Jeffery, J. Bassett, J. Mendham and R. C. Denney, Longman Scientific & Technical Co-published in the United State with John Wiley & Sons, Inc., New York, (1989), 302-303.

[38] L. Wang and A. Wang, Adsorption behaviours of Congo red on the N, O-carboxymethylchitosan/montmorillonite nanocomposite, The Chemical Engineering Journal 143 (2008), 43-50.

[39] Y. B. Wub, S. H. Yue, F. L. Mia, C. W. Wub, S. S. Shyuc, C. K. Pengb and A. C. Chao, Preparation and characterization on mechanical and antibacterial properties of chitosan/cellulose blends, Carbohydrate Polymers 57 (2004), 435-440.

[40] X. Xi-Guang Chen and H. Park, Chemical characteristics of O-carboxymethyl chitosans related to the preparation conditions, Carbohydrate Polymers 53 (2003), 355-359.

[41] T. Xu, M. Li, H. Huang and S. Zhou, Synthesis, characterization and antibacterial activity of N,N,N-trimethyl chitosan and its carboxymethyl derivatives, Carbohydrate Polymers 81 (2010), 931-936.

[42] Y. X. Xu, K. M. Kim, M. A. Hanna and D. Nag, Chitosan-starch composite film: Preparation and characterization, Industrial Crops and Products 21 (2005), 185-192.

[43] J. Yin, K. Luo, X. Chen and V. V. Khutoryanskiy, Miscibility studies of the blends of chitosan with some cellulose ethers, Carbohydrate Polymers 63 (2006), 238-244.

[44] A. Zamani, D. Henriksson and M. J. Taherzadeh, A new foaming technique for production of super absorbents from carboxymethylchitosan, Carbohydrate Polymers 80 (2010), 1091-1101.

[45] L. Zhang, J. Guo and Y. Du, Morphology and properties of cellulose/chitin blends membranes from NaOH/thiourea aqueous solution, Journal of Applied Polymer Science 86 (2002), 2025-2032.

[46] H. Zheng, J. Zhou, Y. Du and L. Zhang, Cellulose/chitin films blended in NaOH/urea aqueous solution, Journal of Applied Polymer Science 86 (2002), 1679-1683.

[47] L. Y. Zheng, J. F. Zhu and K. S. Sun, Antimicrobial activity of chitosan, Materials Science and Engineering 18(2) (2000), 22-24.

[48] A. Zhu, M. B. Chan-Park, S. Dai and L. Li, The aggregation of O-carboxymethylchitosan in dilute aqueous solution, Colloids and Surface B: Biointerface 43 (2005), 143-149.

[49] X. P. Zhuang and X. F. Liu, Blend films of O-carboxymethylchitosan and cellulose in N-methylmorpholine-N-oxide monohydrate, Journal of Applied Polymer Science 102 (2006), 4601-4605.

[50] Z. Cai and J. Kim, Characterization and electromechanical performance of cellulose-chitosan blend electroactive paper, Smart Mater. Struct. 17 (2008), 1-8.

[51] J. Zhou, Y. Du and L. Zhang, Cellulose/chitin films blended in NaOH/urea aqueous solution, Journal of Applied Polymer Science 86 (2002), 1679-1683.

■