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Preparation, characterization and antibacterial properties of cyanoethylchitosan/cellulose acetate polymer blended films

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ABSTRACT

Modification of chitosan (CS) by means of blending with other polymers is a convenient method to improve its physical properties for practical utilization. The similarity of cellulose and chitosan in primary structures might facilitate the formation of homogeneous films. Mixing cyanoethylchitosan (CECS) with cellulose acetate (CA) overcomes mechanical weakness of CS films. CECS is prepared by reaction of chitosan with acrylonitrile in the vapour phase under a different reaction conditions, namely, sodium hydroxide concentration and reaction period. Blended films of CECS and CA are made by casting from dimethylformamide (DMF) followed by impregnation in water. CECS/CA films obtained are characterized by nitrogen content, tensile strength, elongation at break, FTIR and thermogravimetric analysis (TGA). Antibacterial activity of films prepared against Gram (−ve) and Gram (+ve) bacteria is measured via both disk diffusion method and bacterial count method. Blended films show good antibacterial effects toward Gram (+ve) bacteria than Gram (−ve) ones. Antibacterial activities of blended films increased by increasing the CECS percent in the blended films while the tenacity of these films gradually decreased. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) show that CECS possesses higher effect on Gram (+ve) bacteria *Staphylococcus aureus* than Gram (−ve) bacteria *Escherichia coli.* In the meantime, CECS is much more powerful in inhibition of bacteria than CS itself.

1. Introduction

Chitosan is a copolymer of glucosamine and N-acetyl glucosamine units linked by 1,4-D-glucosidic bonds (Isogai & Atalla, 1992; Urreaga & Orden, 2006). It has good biocompatibility, biodegradability, nontoxic 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, membranes, hollow fibers, drug delivery system, etc. (Basavaraju, Damappa, & Rai, 2006; Liu, Chen, & Pan, 2007; Rogovinaa, Akopovab, Vikhorevac, & Gorbachevac, 2001; Shih, Shieh, & Twu, 2009; Twu, Huang, Chang, & Wang, 2003; Urreaga & Orden, 2006; Wub et al., 2004; Zhang, Guo, & Du, 2002; Ma et al., 2008; Zhuang & Liu, 2006) 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, cyanoethylchitosan, which is a kind of derivative containing a –CN group at C-6 position, shows stronger antibacterial activities and better solubility in organic solvents e.g. trifluoroacetic acid, m-cresol, formamide, N,N-dimethylformamide, N,N-dimethyl sulfoxide and showing cholesteric phase to be used in filtration, dialysis and insulating papers due to its biodegradability (Andriyanova et al., 2006; Dong, Yuan, & Huang, 2000; Dong, Yuan, Wu, & Wang, 2000a, 2000b; Dung & Li, 1998; Lee, Shin, & Noh, 1991; Muzzarelli, 1983; Nada, El-Sakhawy, Kamel, Eid, & Adel, 2006).

Cellulose is a poly-β-1,4-D-glucopyranose and it is biodegradable, nontoxic, biocompatible, hydrophilic, safe, has high moisture-retentivity and chiral. 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; also, it has many uses as emulsifier, stabilizer, dispersing agent, thickener, and gelling agent for the preparation of textiles. However, cellulose has not reached its potential application in many areas because of its infusibility and insolubility (Isogai & Atalla, 1992; Liu et al., 2007; Wub et al., 2004; Zhang et al., 2002). Introduction of some amino groups into a cellulosic system is important to facilitate chemical modification. However, it is

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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, 1992). 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, 1992; Liu et al., 2007; Twu et al., 2003; Zhang et al., 2002).

The 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 favored by formation of intermolecular hydrogen bonds between the component polymers (Basavaraju et al., 2006; Zhang et al., 2002; Yin, Luo, Chen, & Khutoryanskiy, 2006). 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., 2001; Twu et al., 2003; Wub et al., 2004). The blends of cellulose and chitosan present special interest; while fibers 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, 2006).

In the present study, new blended films of cyanoethylchitosan and cellulose acetate have been prepared and characterized. Antibacterial tests are studied to examine the possibility of CECS/CA blended films to be used as a biomaterial for wound dressing.

2. Experimental works

2.1. Material

The polymers used are CS (Aldrich, viscosity 1860 cps, deacetylation 79.0%) and CA (Aldrich, Acetyl content 39.7 wt%, Average Mn ca. 50,000). Sodium hydroxide (Modern Lab chemicals, Egypt), acrylonitrile (Merck-Schuchardt, Germany) are used without further purification. Methyl alcohol, ethyl alcohol, acetic acid and isopropyl alcohol (Sisco Research Laboratories, India) and carbon disulfide (Fluka, Germany) and all other chemicals used are analytical grade.

2.2. Methods

2.2.1. Cyanoethylation of chitosan

Alkali-chitosan method was employed to prepare CECS (Tokura, Nishi, Nishimura, & Ikeuchi, 1983) with slight modification where CS is first dissolved in acetic acid to yield 1% aqueous acetic acid solution which might be filtered if necessary using a filter cloth followed by neutralization in aqueous sodium hydroxide under good stirring. The resultant fine powder is collected and mixed in different flasks containing 2–30% aqueous sodium hydroxide followed by filtration and the filter cake is pressed up to 100% wet-pick up. The resultant cake is treated in soxelet apparatus with acrylonitrile vapours for different periods of time. The prepared cyanoethylchitosan was washed with methanol, dried and analyzed for nitrogen content to determine the degree of substitution (DS). The obtained cyanoethylchitosan is soluble in dimethylformamide (DMF), dimethylsulphoxide (DMSO), trifluoroacetic acid (TFA) and acetone. Sodium hydroxide concentration and reaction time were studied in details to determine best reaction yield.

2.3. CECS/CA blended film

CA and CECS are dissolved in DMF separately (8.0 wt%). CA and CECS solutions are blended together in different ratios. Transparent films were obtained by casting these solutions to produce blends with final compositions 9/1, 8/2, 7/3, 6/4, 5/5, 4/6, 3/7, 2/8 and 1/9 (w/w). The hard films remained after evaporation of solvent are soaked in distilled water at room temperature for 1 day to separate blended films from the plate. They are then washed with water, and dried at 50 °C temperature.

2.4. Analysis

2.4.1. Nitrogen content determination

Nitrogen content was determined using micro-Kehjeldal Procedure (Vogel, 1989).

2.4.2. Tensile properties

Tensile properties for the blended films with thickness 55 μm were measured according to ASTM-D6693-01 standard method (LR 5K, LLOYD, England).

2.4.3. FTIR spectroscopy

The FTIR spectra of the samples were recorded by using an FT-IR spectrophotometer (Nexus 670, Nicolet, USA) in the region of 4000–400 cm^{-1} with spectra resolution of 4 cm^{-1}.

2.4.4. Thermal gravimetric analysis

The thermal analysis experiments were conducted using simultaneous thermal gravimetric analyzer (Perkin Elmer thermogravimetric analyzer, TGA7, the heating rate is 10 °C/min, USA).

2.5. Evaluation of antibacterial activity in vitro

2.5.1. Materials

Four bacterial strains from the Faculty of women for Art, Science and Education, Ain Shams University, Cairo, Egypt were employed. They include Gram-positive (G +ve) bacteria: *Staphylococcus aureus* (*S. aureus*) and *Bacillus subtilis* (*B. subtilis*) and the Gram-negative (G −ve) bacteria: *Escherichia coli* (*E. coli*), and *Proteus*. For MIC, MBC and bacterial counts *S. aureus* and *E. coli* were selected as test cells because they are the most frequent bacteria in the wound infection to represent G +ve and G −ve bacteria, respectively. Fresh inoculants for antibacterial assessment were prepared on nutrient broth at 37 °C for 24 h.

2.5.2. Test methods

2.5.2.1. MIC and MBC method for modified chitosan. To assess the antimicrobial activity of the CS and CECS, MIC and MBC were evaluated according to broth tube dilution method (Mazzola, Jozala, Novaes, Moriel, & Penna, 2009).

2.5.2.2. Disk diffusion method for CECS/CA blended films. The antibacterial spectrum of CECS/CA blended films was determined against the test bacteria by disk diffusion method on an agar plate (Miller et al., 2003).

2.5.2.3. Bacterial count method for CECS/CA blended films. The antimicrobial activity of CECS/CA blended films are determined against the tested bacteria by colony counting method (Chung & Chen, 2008). The percentage reduction was determined as follows:

$$\text{Reduction in CFU (colony forming units)\%} = \frac{(C - A)}{C} \times 100$$

where *C* and *A* are the colonies counted from the plate of the control and treated samples, respectively.

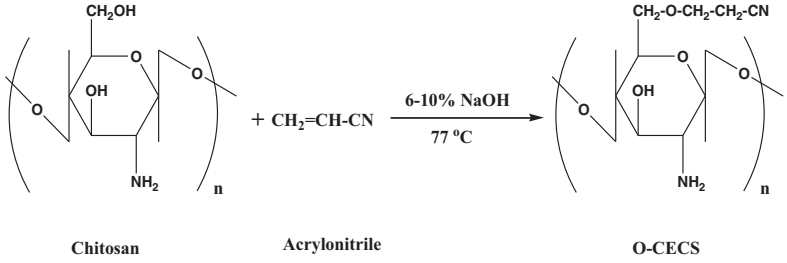
In the antibacterial assay all data were the means from at least three parallel experiments that the discrepancies among them were <5%.

3. Results and discussion

3.1. Cyanoethylchitosan of chitosan

To study the antimicrobial behaviour of cyanoethylchitosan alone or in admixture with cellulose acetate, cyanoethylchitosan has been prepared as described in Section 2. Table 1 shows the effect of sodium hydroxide concentration and reaction period on the degree of substitution (DS) and solubility of the resultant cyanoethylchitosan (CECS) in DMF.

It is clear from Table 1 that keeping the aqueous sodium hydroxide concentration in the filter cake between 6 and 10% is quite proper to activate the double bonds of acrylonitrile to facilitate its addition on the hydroxyl groups of chitosan.



Sodium hydroxide concentration <6% is not enough to activate the double bonds, while concentration ≥10% is enough to activate the double bonds and to attack the nitrile groups to yield amide and/or carboxyl groups (Hebeish & Khalil, 1988; Sharma, Kumar, & Soni, 2003). The results of solubility confirm this result where a DS <1.2 is not enough to open the structure and the material swells only while at a DS 1.20–1.23 the structure is open enough to let the material dissolve in DMF. Increasing the sodium hydroxide concentration above 10% will facilitate the hydrolyzing attack of NaOH on the CN group to convert it to $-CONH_2$ and/or –COOH which are capable of closing the structure again by hydrogen bonding.

It is clear from Table 1 that keeping NaOH concentration at 6% aqueous level yields a wide range of reaction periods between 2 and 6 h through which the resultant CECS remains soluble in DMF. Increasing the reaction period up to 9 h or more yields a product which is insoluble in DMF a result which might indicate that using 6% aqueous NaOH and keeping the reaction period at a minimum of <2 h and for a maximum of <9 h will yield a soluble CECS product in DMF.

3.2. Characterization of cyanoethylchitosan/cellulose acetate blended films

3.2.1. Nitrogen content and mechanical properties

Cellulose acetate and cyanoethylchitosan are dissolved in DMF separately to yield 8.0 wt% solutions. These solutions are mixed together in different ratios. Transparent films were obtained by casting these solutions to produce blends with final compositions 100% CA: 0.00% CECS to 0.00% CA: 100% CECS (ratios in between are 9/1, 8/2, 7/3, 6/4, 5/5, 4/6, 3/7, 2/8 and 1/9 (w/w) respectively). The hard films remained after evaporation of solvent are soaked in distilled water at room temperature for 1 day to separate them from the glass-plate followed by washing with water and dried at 50 °C. Table 2 shows some chemical and physical properties of the resultant CA/CECS blended films.

It is clear from Table 2 that blending the two polymers together yields more or less homogeneous films as it is shown by the stepwise increase in the nitrogen content and the elongation at break% and also by the stepwise decrease in the tensile strength of the resultant films by increasing the CECS content. Homogeneity of blended films is discussed and clarified in details as described under TGA analyses.

Table 1
Effect of the sodium hydroxide concentration and reaction period on DS of resultant cyanoethylchitosan and its solubility in DMF.

Factor studied		Nitrogen content *N*%	DS of cyanoethyl groups	Solubility in DMF
[Sodium hydroxide] %	2	11.66	0.84	Swell
	4	12.46	1.05	Swell
	6	13.10	1.20	Soluble
	8	13.01	1.23	Soluble
	10	12.97	1.19	Soluble
	12	11.09	0.69	Swell
	15	10.91	0.64	Swell
	20	10.48	0.52	Insoluble
	25	10.42	0.51	Insoluble
	30	9.44	0.24	Insoluble
Reaction period (hrs)	0.5	10.83	0.62	Insoluble
	1.0	11.42	0.78	swell
	2.0	12.42	1.05	soluble
	3.0	13.10	1.32	soluble
	4.0	12.55	1.08	soluble
	5.0	12.30	1.01	Soluble
	6.0	11.55	0.81	soluble
	9.0	11.30	0.74	insoluble
	12.0	10.73	0.59	insoluble

Experimental conditions used: [chitosan], 5 gm; [acrylonitrile], 25 ml in vapour phase; reaction period, 3 h; [NaOH] 6%; wet pick-up of mother liquor of NaOH reaches 100%.

Table 2
Percent nitrogen content, tensile strength and elongation yield% of CA/CECS and Viscose/CMCS blended films.

	Film composition	Nitrogen content N%	Tensile strength Pa	Elongation yield %
CA: CECS	100:0	0.00	6.80	3.02
	90:10	1.18	6.48	3.49
	80:20	1.61	6.00	3.68
	70:30	2.30	5.68	4.53
	60:40	2.77	5.32	4.74
	50:50	4.12	5.10	5.01
	40:60	6.22	4.84	5.41
	30:70	7.01	4.77	5.67
	20:80	8.94	4.42	6.47
	10:90	12.00	4.09	7.60
	0:100	13.10	3.38	8.21

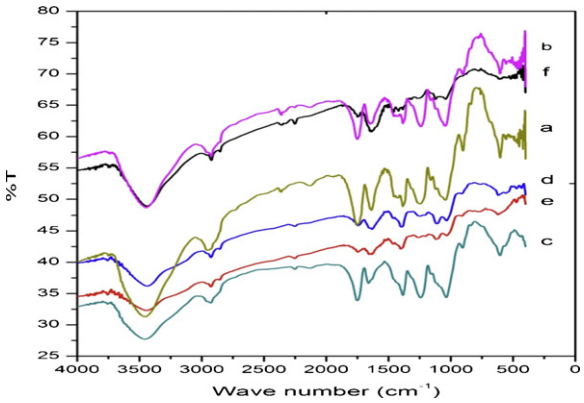


Fig. 1. FTIR spectra of CA film (a), CA:CECS blended films 80:20 (b), 60:40 (c), 40:60 (d), 20:80 (e) and CECS film (f).

3.2.2. FTIR analysis

The FTIR transmission spectra measured for all blended films shows absorption maxima at approximately the same wave number indicating that mixing the cyanoethylchitosan and cellulose acetate yield a regular polymer structure with a slight shift in the absorption maxima. Films prepared from CECS and cellulose acetate Fig. 1a–f are transparent and flexible. The data on infrared spectra of the pure component and the blends are summarized in Table 3 and are shown in Fig. 1. The bands at 3442 in CECS and at 3452 in CA can be due to the OH-stretching which overlaps with NH-stretching in the same region (NH-absorption band at 3260 cm^{-1}). The peaks at 2918 and 2933 for both spectrum corresponds to CH-stretching. Moreover, the absorption band at 2247 cm^{-1} in spectrum (f) corresponding to –CN stretching diminishes by decreasing CECS in the blend. This band is overlapped in case of 80:20, CA:CECS which once more might be a sign for good miscibility. The bands at 1748, 1640 cm^{-1} for CECS and at 1751 and 1633 cm^{-1} for CA are C=O stretching. By mixing the two polymers at ratios 80:20 (b), 60:40 (c), 40:60 (d) and 20:80 (e) these absorption maxima are shifted by very few wave numbers from pure polymers. On the other hand, absorption maxima at 2933 cm^{-1} for CA (100%) corresponding to OH-stretching splits to absorption bands at 2961 cm^{-1} and 2925 cm^{-1} by mixing with CECS. The absorption band at 2846 cm^{-1} in CECS (100%) decreases in intensity by decreasing CECS content of the film from 100% to 80% to 60% and by 40% CECS the band is overlapped by the CH-stretching band at 2933 cm^{-1}, an observation which might indicate good miscibility.

In general, it could be concluded that the comparison between spectra of CECS and blended films with CA 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 place between cellulose and chitosan derivatives in the blended films, leading to a good miscibility of the blends.

3.2.3. Thermogravimetric analysis (TGA)

The results of thermogravimetric analysis of CECS/CA blends are given in Fig. 2. The pure CECS film thermal degradation consists of three stages. The first stage is weight loss of up to 2% with a maximum rate at 70.6 °C which is related to the evaporation of water present in the sample. The second stage is weight loss of CA 2.7% with maximum rate at 193.2 °C which is related to crystalline water. The third stage starts at 260 °C with maximum at 343.6 °C corresponding to a weight loss of CA 61.2% this temperature is related to the depolymerization of CECS chains. This degradation profile of CECS is in good agreement with the results of chitosan degradation reported by Wanjun, Cunxin, and Donghua (2005). The pure CA shows also a two stage thermal degradation. The first of which lies at 60 °C with weight loss of up to 5%. The second stage starts at 220 °C with maximum at 318.1 °C corresponding to weight loss of 66.4%, this temperature is related to the depolymerization of CA chains. By blending CECS and CA in ratios 80:20, the maximum weight loss of 59.7% takes place at 336.1 °C; for a ratio 60:40 first weight loss of 13.5% takes place at 214.6 °C and a second weight loss of 55.5% takes place at 337.5 °C; for a ratio 40:60 two stages are there the first is minor and the second stage is major and starts at

Table 3
FTIR absorption bands in CA/CECS blends.

Blend CA:CECS (wt%)						
0:100	20:80	40:60	60:40	80:20	100:0	Assignment
3442	3447	3434	3450	3442	3452	–OH and –NH stretching vibration
2918	2927	2925	2961	2958	2933	–CH stretching
2846	2854	2854	2925	2918		
2247	2249	2250	2250	–	–	–CN stretching
1748	1744	1740	1751	1751	1751	–C=O stretching (amide I), in amorphous region
1640	1650	1633	1658	1646	1633	
1124	1109	1251	1241	1238	1248	Anti-symmetric stretching of C–O–C bridge
1037	1030	1035	1033	1044	1044	Skeletal vibrations involving the C=O stretching

ca 200 °C with a maximum at 343.1 °C corresponding to weight loss of 55.1%; for a ratio of 20:80 the thermal disintegration takes place in many stages. Based on these findings, it can be state that mixing CECS with CA at ratios of 80:20, 60:40 and 40:60 yields miscible blends while a mixture based on 20:80 ratio is immiscible.

3.3. Antibacterial properties

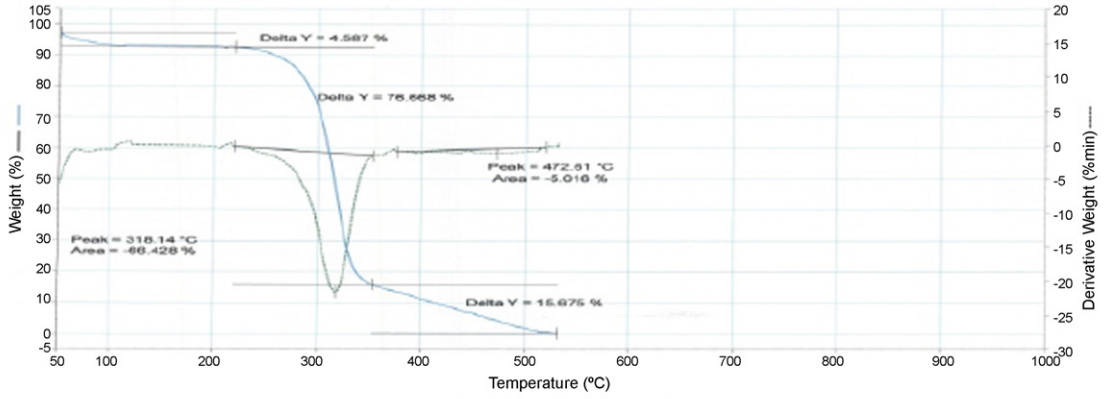
3.3.1. MIC and MBC for CS and CECS

Table 4 shows the antibacterial activity (MIC and MBC) of the chitosan and its derivative CECS, according to the two-fold broth tube dilution method, on *S. aureus* and *E. coli* as described in Section 2.

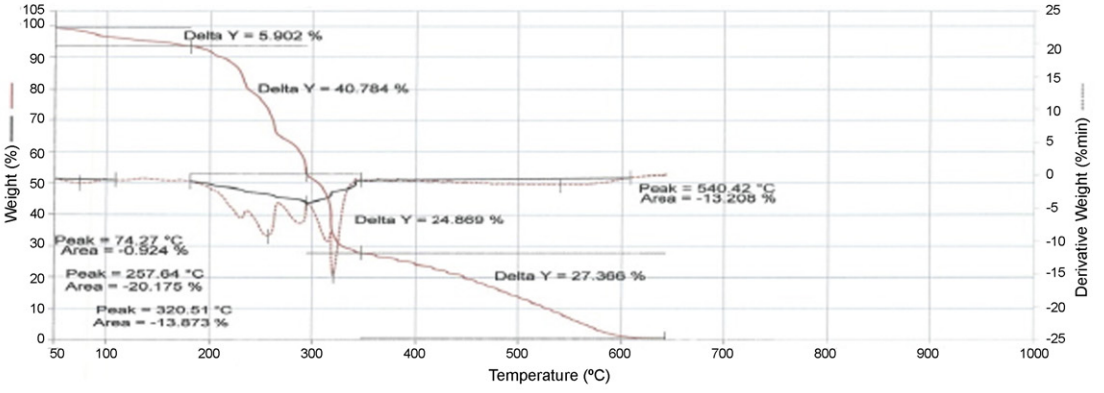
Table 4
MIC and MBC values found for Chitosan, Cyanoethylchitosan.

	S. aureus		*E. coli*	
	MIC (ppm)	MBC (ppm)	MIC (ppm)	MBC (ppm)
CS	78	156	625	2500
CECS	19.5	78	312	1250

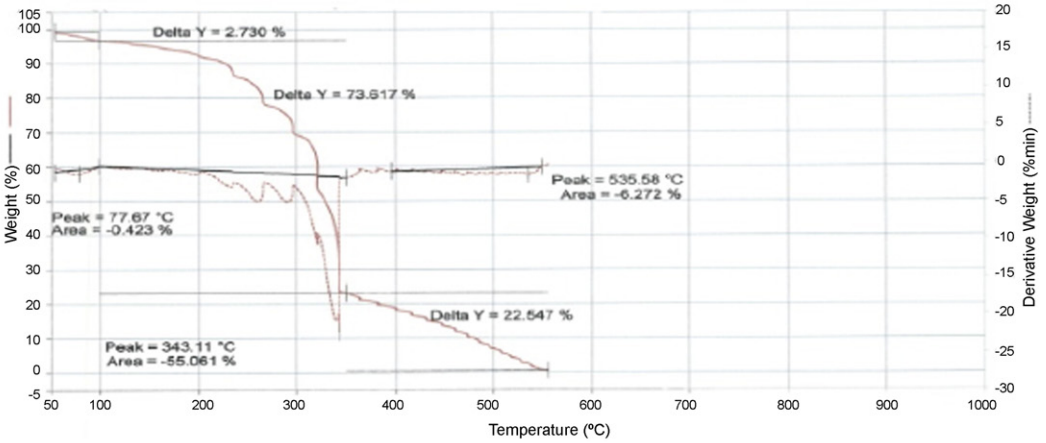
Chitosan, a cationic antibacterial agent, has been widely used, particularly for wound dressing, and the target site of the cationic biocides is the cell envelope of bacteria. The mechanism of antibacterial activities of chitosan that the amino group of chitosan is bound to surface components of the bacteria and then inhibits



a. CECS: CA, 0: 100

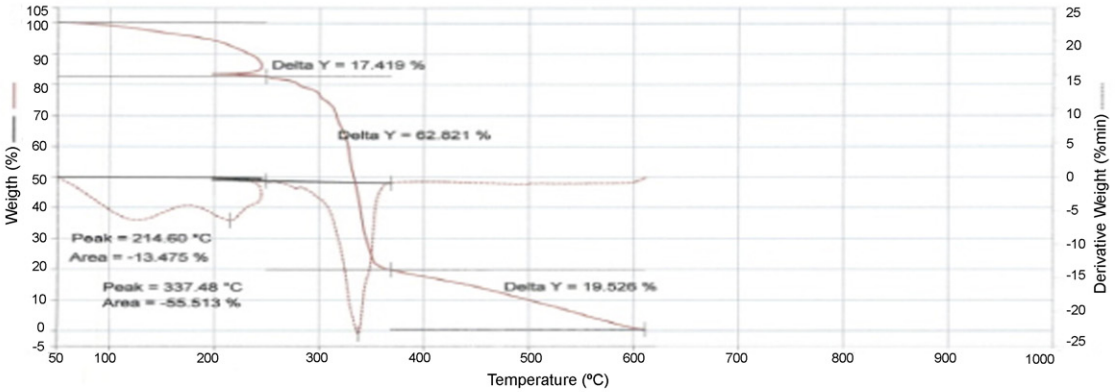


b. CECS: CA,20: 80

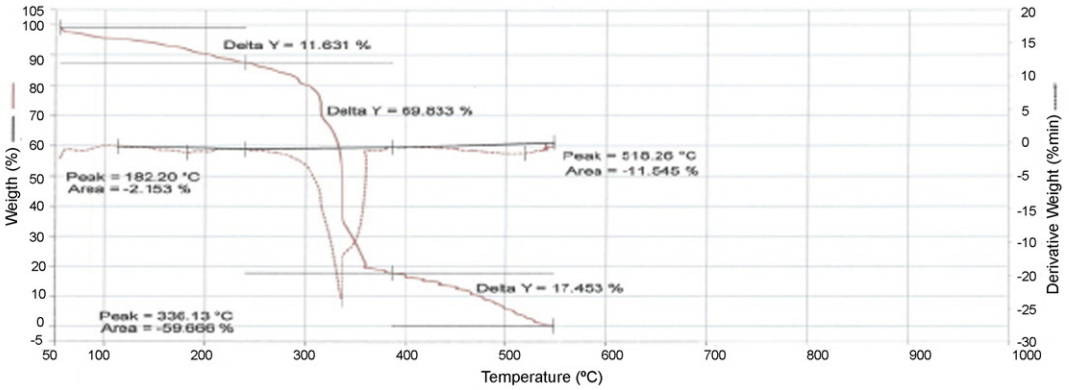


C. CECS: CA, 40: 60

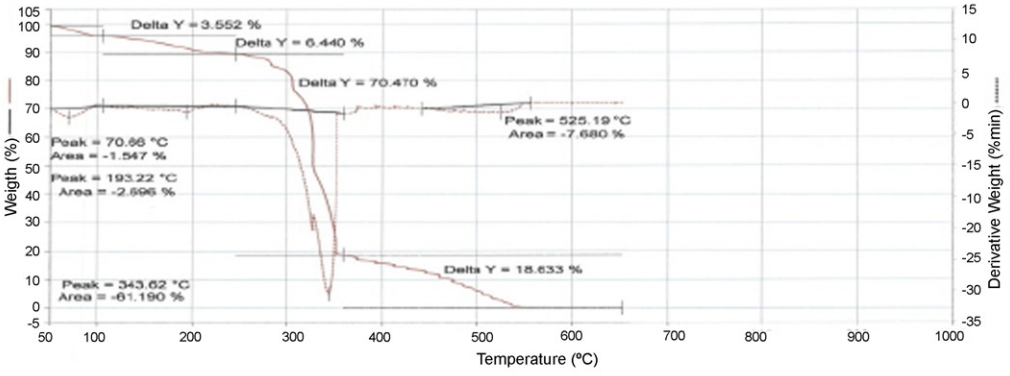
Fig. 2. TGA for CECS/CA polymer blends.



d. CECS: CA, 60: 40



e. CECS: CA, 80: 20



f. CECS: CA, 100:0

Fig. 2. (*Continued*).

their growth was developed (Papineau, Hoover, Knorr, & Farkas, 1991; Sudharshan, Hoover, & Knorr, 1992). They thought that at lower concentration chitosan may have bound to the negatively charged bacterial surface to disturb the cell membrane and cause cell death due to leakage of intracellular components (Zheng, Zhu, & Sun, 2000), at high concentration, chitosan may have additionally coated the bacterial surface to prevent leakage of intracellular components as well as to impede mass transfer across the cell barrier (Helander, Nurmiaho, Ahvenainen, & Roller, 2001).

Antibacterial activity of chitosan, which is a polycationic compound due to a large amount of NH_3^+ in the solution and depend on the concentration of the $-NH_2$ of the polymer. CECS show higher MIC and MBC than chitosan itself due it is the substitution of chitosan with CH_2CH_2CN onto –OH group; where the number of $-NH_2$ groups is not changed and in the meantime it has CN groups with high killing effect for bacteria.

CS and CECS had more effective inhibition on *S. aureus* than *E. coli* (Chung et al., 2004; Sun, Du, Fan, Chen, & Yang, 2006; Xu, Li, Huang, & Zhou, 2010). The fact may be attributed to their different cell walls. *S. aureus*, a typical 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. On the other hand, the cell wall of *E. coli*, a typical Gram-negative bacterium 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., 2008; Sun et al., 2006).

Table 5
Inhibition zones and percent reduction in bacterial growth of CA/CECS bended polymer discs of different polymer ratios and tested against G +ve and G −ve bacteria.

CA:CECS polymer ratio	Diameter of clear zone (mm)				Reduction (%)	
	Gram positive		Gram negative			
	S. aureus	*B. subtilis*	*E. coli*	*Proteus*	*S. aureus*	*E. coli*
100:0	0	0	0	0	6.8	5.2
90:10	0	0	0	0	44.7	15.5
80:20	0	0	0	0	59.3	25.0
70:30	0	0	0	0	57.1	24.5
60:40	0	0	0	0	99.3	25.9
50:50	0	15	0	0	99.4	33.9
40:60	15	14	0	0	99.9	43.4
30:70	13	15	0	15	99.7	51.8
20:80	17	15	14	14	99.9	62.2
10:90	16	15	14	16	99.9	68.9
0:100	16	15	16	19	99.9	76.8

3.3.2. Disk diffusion and bacterial count methods for CA/CECS polymer films

The antibacterial activity of modified chitosan and cellulose polymer blends are tested against Gram-negative bacteria and Gram-positive bacteria by the disk diffusion standard method and colony counting methods as mentioned in Section 2.

Table 5 illustrates that blended films of CA:CECS shows no resistance to growth of bacteria (both G +ve and G −ve) as long as the ratio CA:CECS is 100:00 to 60:40. Blended films having a ratio 50:50 succeeded to inhibit growth of G +ve *B. subtilis* only. Films having a ratio of 40:60 possess inhibition properties for both G +ve *S. aureus* and *B. subtilis*. Changing the ratio to 30:70 inhibition is positive for both G +ve staph and *B. subtilis* and Proteus from the G −ve group. Films having constitution ratio of 20:80, 10:90 and 00:100 possess inhibition of growth for both G +ve and G −ve bacteria used. It is clear from Table 5 that CA:CECS blended polymer ratio of 60:40 is enough to reduce growth of *S. aureus* by 99% and by increasing the chitosan derivative content up to 00:100 CECS the film shows a gradually increase in reduction up to ca. 99.9%. In case of *E. coli* the percent reduction gradually increased from 5.2% up to 76.8% by changing blended polymer ratio from 100:00 to 00:100.

4. Conclusion

- Cyanoethylation of chitosan was carried out with acrylonitrile in the vapour phase under a variety of reaction conditions, namely, sodium hydroxide concentration and reaction period. The optimum reaction conditions for cyanoethylation of chitosan are: [NaOH] is 6%; and reaction period is 3 h.
- MIC and MBC for CECS in DMSO more than CS in acetic acid due to the presence of CN groups that has high killing effect.
- CECS/CA blended were prepared successfully by mixing CECS and CA in DMF as co-solvent, and impregnated in water.
- The mechanical properties of CECS films were improved after incorporation of CA into the blended films and the antibacterial activity of the blended films increased by increasing the CECS percent in the blended films.
- The miscibility of CECS/CA bended films has been assessed by nitrogen content, FTIR and TGA analyses.
- CECS/CA blended films impart good antibacterial effects toward G (+ve) than G (−ve) bacteria due to the structure of bacteria cell wall, and they can used as wound healing due to their antibacterial effects.

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