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Review

Comparative multifunctional properties of partially carboxymethylated cotton gauze treated by the exhaustion or pad-dry-cure methods

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ABSTRACT

A commercial cotton gauze was modified by partial carboxymethylation using both exhaustion and pad-dry-cure methods, and varying the reaction time and concentration of monochloroacetic acid and sodium hydroxide to obtain the relative degree of carboxymethylation differently. For each experiment, relative value of the degree of substitution (DS_{rel}) of the modified cotton was evaluated and compared with whole blood clotting time, absorption and retention of chitosan and silver nitrate solutions, antibacterial activity, and physical properties of whiteness, bursting strength and water absorption. Carboxymethylated cotton gauze with a higher DS_{rel} value showed a better absorption of chitosan and silver nitrate solutions and retained these two solutions for a much longer time than those of unmodified cotton gauze or carboxymethylated cotton gauze at a lower DS_{rel}. Carboxymethylated cotton gauzes obtained from exhaustion method showed significant antibacterial activity and higher bursting strength and less affected whiteness index than those treated by pad-dry-cure method.

Contents

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1. Introduction

Cotton gauze is a type of thin cotton fabric with a very open weave made of cellulose fibers that is principally designed for medical wound dressing and as such has become a very useful and universal material in first aid kits. With respect to medical wound dressings it consists of three important parameters; (1) keeping the wound clean by protecting it from infection, (2) keeping the wound moist by maintaining a moist environment at the wound site to enhance healing and prevent or reduce a maceration condition, and (3) keeping the wound well nourished by absorbing fluid exudates and toxic components (Bezzant, 2010). Cotton gauze is a highly absorbent material by itself and, therefore, can be used to take up blood, plasma and other fluids from open wounds very effectively. Therefore, it is standard practice to use cotton gauze as a first aid tool to cover open wounds, assuage bleeding on wounds and to pad wounds before they are bandaged. However, the disadvantage of cotton gauze is the lack of ability to protect the open wound from infection and bacterial contamination. This drawback could be solved by chemical modification on the cotton gauze, including through a carboxymethylation process (Racz, Borsa, & Bodor, 1996; Racz, Deak, & Borsa, 1995; Hashem, Refaie, & Hebeish, 2005). In these previous research articles, the methods used to modify virgin cotton to partially carboxymethyl cotton were exhaustion and pad-roll methods. The reaction of carboxymethylation of cotton gauze was produced by the interaction of the hydroxyl groups of cotton gauze with monochloroacetic acid in the presence of aqueous alkali (Heinze & Pfeiffer, 1999). Degree of substitution reflected the average number of carboxymethyl groups per anhydroglucose unit of cellulose. When cotton was carboxymethylated to a degree of substitution (DS) of 0.2 or less using monochloroacetic acid and sodium hydroxide, it was found to have considerably altered properties from that of the unmodified cotton (Parikh et al., 2005; Parikh, Sachinvala, & Calamari, 2003; Pushpamalar, Langford, Ahmad, & Lim, 2006). These included an increased wettability, moisture regain and water solubility, changed dyeing characteristics, improved ease of soil removal and an increased reactivity to further chemical treatment allowing the production of an antimicrobial cloth, which are all advantageous properties.

The objective of this research was to modify cotton gauze by partial carboxymethylation using sodium hydroxide and monochloroacetic acid applied by the exhaustion and the pad-dry-cure methods for a direct comparison between the two methods. The pad-dry-cure method was selected to study for a comparison with the exhaustion method because the pad-dry-cure method has been widely used in the textile finishing. Three reaction factors, namely the reaction time and the concentration of monochloroacetic acid and sodium hydroxide were independently varied so as to affect the relative degree of substitution (DS_{rel}) of the carboxymethylation-modified cotton gauze . The ability to modify the whole blood clotting rate and the antibacterial properties, the absorption and retention of 0.5% (w/v) chitosan and silver nitrate solutions, water absorption, whiteness and the bursting strength of the different preparations of unmodified and modified cotton gauzes were evaluated and related to their respective DS_{rel} values and different treating methods.

2. Experimental

2.1. Materials

2.1.1. Cotton gauze

Mill scoured and bleached loose plain weave cotton gauze having the warp density of 21 yarns/cm and weft density of 19 yarns/cm, was supplied by Limmer Co. Ltd., Thailand.

2.1.2. Chemicals used

Hydrochloric acid, acetic acid and ethanol were purchased from J.T. Baker Co. Ltd. Sodium hydroxide, isopropanol, monochloroacetic acid, and silver nitrate were purchased form Ajax Finechem, SK Chemicals, Sigma–Aldrich and POCH SA Co. Ltd., respectively. All chemicals used in this study were all analytical reagent grades. A commercial grade of chitosan having a MW of 480 kDa and a 90% degree of deacetylation was supplied by Bio-Line Co. Ltd., Thailand.

2.2. Preparation of partially carboxymethylated cotton gauze

2.2.1. Exhaustion method

A 20 g sample of cotton gauze was weighed and placed into a 1000 ml beaker and then 400 ml of an 80% (v/v) isopropanol in water solution was added and stirred for 30 min at ambient temperature. To this, 100 ml of 40% (w/v) sodium hydroxide was added drop-wise and stirred for another 30 min at ambient temperature. The carboxymethylation reaction was started with the addition of 100 ml of a 15% (w/v) monochloroacetic acid dissolved in isopropanol. The mixed solution was heated up to 70 °C with constant mixing for 3 h (the reaction time), after which the modified cotton gauze was taken out, washed by soaking in water, and then neutralized by the addition of 0.5 M HCl until the pH reached 7. The modified cotton gauze was then removed, washed again with an excess of 90% (v/v) ethanol in water, and then dried at 70 °C for 1 h. The above protocol was amended to allow the independent variation of the reaction time (3 and 4 h) and the concentration of sodium hydroxide (40% and 45% (w/v)) and monochloroacetic acid (15%, 20%, 25% and 30% (w/v)), so as to assay all 16 permutations (see Table 1). However, the initial accepted parameters were that it should not cause any gelling characteristics on the modified cotton gauze.

2.2.2. Pad-dry-cure method

Solutions of 200 ml of aqueous sodium hydroxide (30% and 35% (w/v)) and 200 ml of monochloroacetic acid (15%, 20% and 25% (w/v)) in isopropanol were prepared. A 20 g piece of cotton gauze was soaked in an excess (400 ml) of one of the six combinations of the mixed sodium hydroxide and monochloroacetic acid solution with two dips (each for 1 min) at room temperature, followed by squeezing to obtain a wet pickup of 70%. Then, the padded cotton gauze was dried at 70 °C for 10 min and cured at 120 °C for 3, 7 or 10 min. After curing, each of the modified cotton gauze preparations (18 different permutations, see Table 2) was neutralized, washed and then dried, with the same initial acceptance criteria, as detailed above (Section 2.2.1).

Table 1
The effect of varying the reaction time and concentration of monochloroacetic acid ($CH_2ClCOOH$) and sodium hydroxide (NaOH) in the exhaustion method on the carboxymethylation DS_{rel} and physical characteristics of the modified cotton gauze.

Independent variable			DS_{rel} [a]	Changed characteristics[b]
$CH_2ClCOOH$ (%)	NaOH (%)	Time (h)		
15	40	3	0.43 ± 0.03	–
20	40	3	0.56 ± 0.03	–
25	40	3	0.68 ± 0.03	–
30	40	3	0.84 ± 0.03	Beginning to become hard to handle
15	40	4	0.44 ± 0.03	–
20	40	4	0.62 ± 0.04	–
25	40	4	0.73 ± 0.02	Beginning to become hard to handle
30	40	4	1.01 ± 0.03	Surface gelling characteristic
15	45	3	0.55 ± 0.03	–
20	45	3	0.63 ± 0.02	–
25	45	3	0.81 ± 0.02	Beginning to become hard to handle
30	45	3	1.12 ± 0.03	Surface gelling characteristic
15	45	4	0.61 ± 0.03	–
20	45	4	0.72 ± 0.02	Beginning to become hard to handle
25	45	4	0.85 ± 0.04	Beginning to become hard to handle
30	45	4	1.41 ± 0.03	Surface gelling characteristic

[a] Data are shown as the mean ± 1 SD and are derived from 5 repeats.
[b] The standard (acceptable) physical characteristic was a retaining gauze fabric that was soft to handle.

2.3. Determination of the relative value of the degree of substitution (DS_{rel}) with carboxymethyl groups

The DS_{rel} of the carboxyl group onto the treated cotton gauze in this study was determined by Thermo Nicolet 6700 FTIR spectroscopy using the ATR technique, where DS_{rel} was estimated from the IR spectra as shown below (Miyamoto, Tsuji, Nakamura, Tokita, & Komai, 1996):

$$DS_{rel} = R_{rel} - 1$$

where R_{rel} is the ratio of the absorption spectra of A_{1605}/A_{2920}.

The original cellulose sample has the DS_{rel} value equal to zero. The FTIR absorption at 1605 cm^{-1} is assigned to the stretching vibration of the carboxyl group (COO^-) and the absorption at 2920 cm^{-1} is assigned to the stretching vibration of methane (C–H) group, respectively. Calculation of the ratio of these two absorption spectra of A_{1605}/A_{2920} leads to the estimated relative amount of carboxyl groups in the tested sample.

2.4. The absorption and retention of a 0.5% (w/v) silver nitrate solution on the different cotton gauzes

Silver nitrate has been used as an antiseptic agent in hospitals for a long time, where a maximum concentration of 0.5% (w/v) silver nitrate provides the best effective antimicrobial activity without damaging the human tissue. Therefore, if the cotton gauze can hold this or near this concentration of silver nitrate for a longer period of time, it could prevent the wound from becoming infected for a longer period of time allowing it to seal without trapping bacteria inside for example. Moreover, the current practice of rewetting

Table 2
The effect of varying the reaction time and concentration of monochloroacetic acid ($CH_2ClCOOH$) and sodium hydroxide (NaOH) in the pad-dry-cure method on the carboxymethylation DS_{rel} and physical characteristics of the modified cotton gauze.

Independent variable			DS_{rel} [a]	Changed characteristics[b]
$CH_2ClCOOH$ (%)	NaOH (%)	Time (min)[c]		
15	30	3	0.12 ± 0.02	–
20	30	3	0.46 ± 0.02	–
25	30	3	0.74 ± 0.01	Beginning to become hard to handle
15	30	7	0.17 ± 0.01	–
20	30	7	0.48 ± 0.02	–
25	30	7	0.95 ± 0.02	Beginning to become hard to handle
15	30	10	0.18 ± 0.01	–
20	30	10	0.55 ± 0.02	–
25	30	10	0.98 ± 0.03	Beginning to become hard to handle
15	35	3	0.24 ± 0.03	–
20	35	3	0.66 ± 0.02	–
25	35	3	1.00 ± 0.04	Beginning to become hard to handle
15	35	7	0.25 ± 0.02	–
20	35	7	0.67 ± 0.02	–
25	35	7	1.04 ± 0.03	Surface gelling characteristic
15	35	10	0.28 ± 0.02	–
20	35	10	0.70 ± 0.02	Beginning to become hard to handle
25	35	10	1.33 ± 0.03	Surface gelling characteristic

[a] Data are shown as the mean ± 1 SD and are derived from 5 repeats.
[b] The standard (acceptable) physical characteristic was a retaining gauze fabric that was soft to handle.
[c] The curing time.

the cotton gauze with fresh 0.5% (w/v) silver nitrate solution every 2 or 3 h may not be required. Thus, the retention of silver nitrate by the different modified cotton gauzes was determined using a modified dunk-and-drain test, as described in Parikh et al. (2005). The test sample was kept at 45 °C in the oven for 24 h before testing. Silver nitrate solution was prepared to 0.5% (w/v) in 85% (v/v) ethanol/15% (v/v) water. Each 2 g piece of test cotton gauze specimen was soaked in the 0.5% (w/v) silver nitrate solution for 30 s. The absorbent capacity was determined by weighing the wet specimen immediately after draining for 30 s and keeping the specimen in the oven at 37 °C. Retention of silver nitrate on the specimen was determined by weighing the specimen before and after drying at 37 °C in the oven at 30 min intervals up to 2 h.

2.5. The absorption and retention of a 0.5% (w/v) chitosan solution on the different cotton gauzes

The absorption and retention level of a 0.5% (w/v) aqueous chitosan solution on the cotton gauze was also measured in the same manner using the modified dunk-and-drain test as mention above (Section 2.4), except that a 0.5% (w/v) chitosan solution in 2% (v/v) acetic acid/98% (v/v) water was used in place of the silver nitrate solution.

2.6. The effect of the different cotton gauzes on the whole blood clotting time (WBCT)

The WBCT was measured using the modified Lee–White method, as outlined in Laffan and Bradshaw (1995). In this study, the modified cotton gauze was crushed using an ultra centrifugal mill and passed through a 0.08 mm sieve, and then the sieved finings were dried in an oven at 45 °C for 24 h before use. A 0.01 g of the test milled gauze sample was added into each of three glass test tubes of 1.5 cm diameter and 10 cm length. Fresh blood was collected from healthy (non-hemophiliac) volunteers using a two-syringe technique. About 1 ml of fresh blood collected in the first syringe was intentionally discarded and 3 ml of fresh blood collected in the second syringe was transferred to the three glass test tubes at a volume of 1 ml of each. The tubes were incubated in a water bath at 37 °C for 5 min and then observed for blood coagulation at 1 min intervals until all three tubes had coagulated. Here, blood coagulation is defined as no blood flow when the glass test tube was inclined at a 45° angle. WBCT was justified as the time from the start of the blood collection until blood coagulation in all three test tubes. The experiment was repeated five times for each tested sample using fresh blood from five donors and taken the WBCT as the average.

2.7. Water absorption on cotton gauze

The water absorption level of each different preparation of cotton gauze was determined by following the standard method of ASTM D4772-09. For this, 1 g of each test specimen was placed in an oven at 45 °C for 24 h, weighed and then soaked in 100 ml distilled water for 1 h at ambient temperature. The test specimens were then removed and hanged to dry under gravity on a rope for 30 s and then weighed immediately to determine the wet weight of the tested specimens. The water absorption is expressed as the percentage increase in the sample weight and was obtained by averaging the values of five specimens.

2.8. Antibacterial activity on each cotton gauze

The antibacterial efficacy of the cotton gauze was assessed according to the standard method of AATCC 100-1999. In this standard test method, the percent bacterial reduction (R) is calculated as per the following equation:

$$R = \frac{A - B}{A} \times 100$$

where A and B are the number of bacteria recovered from the inoculated tested specimen (by washing) immediately after inoculation and after incubation for 24 h, respectively. The Gram-positive *Staphylococcus aureus* (ATCC 6538) and Gram-negative *Escherichia coli* (ATCC 4352) were used as the test microorganisms.

2.9. Physical properties of the cotton gauze

The physical properties of each of the different cotton gauze samples were characterized in terms of their bursting strength and whiteness index. The bursting strength was measured following the standard method of ASTM D 3786-08, using a bursting strength tester CY-6103A1. The whiteness index was measured according to the standard method of ASTM E313-05, using a Macbeth Color-eye 7000 spectrophotometer.

3. Results and discussion

3.1. Chemical structure of the unmodified cotton gauze and those modified by partial carboxymethylation

Cotton gauze was carboxymethylated with a mixture of monochloroacetic acid and sodium hydroxide solutions by both the exhaustion and the pad-dry-cure methods. Fig. 1 shows the IR spectra of the original unmodified cotton gauze and representative FT-IR spectrum of cotton gauze carboxymethylated with the exhaustion or the pad-dry-cure method for confirming the occurrence of carboxymethylation on the cotton gauze. The appearance of a new absorption band at 1605 cm^{-1}, found only in the modified cotton gauze samples is attributed to the COO^- group. The broad absorption band at 3300 cm^{-1} is due to the stretching vibration of –OH group and that at 2920 is assigned to C–H stretching vibration. These latter two absorption bands appear in both the original (unmodified) and carboxymethylated-modified cotton gauzes.

3.2. The degree of carboxymethylation (DS_{rel}) and physical characteristic of the modified cotton gauze prepared by the different reaction conditions

The unmodified cotton gauze and those modified by carboxymethylation using either the exhaustion method (Table 1) or the pad-dry-cure method (Table 2) were evaluated for their DS_{rel} values and physical acceptability. Both preparation methods could be used to modify the cotton gauze to become partially carboxymethylated. Moreover, in both preparation methods by individually varying the reaction time and the concentration of monochloroacetic acid and sodium hydroxide, different values of DS_{rel} of partially carboxymethylated cotton gauzes were obtained. In general, any of these three variables increased whilst keeping the other two variables constant enhanced the DS_{rel} of the modified cotton gauze. However, when the three parameters were all used at the upper extremes (within the tested ranges) at the same time, the surface of the modified cotton gauze gelled and could not retain the original structure of the gauze fabric. Indeed, the surface of the cotton gauze surface appeared to take on a gelling characteristic when the DS_{rel} value was larger than 1 (Tables 1 and 2).

Using the exhaustion method for carboxymethylation of the cotton gauze samples, the maximum of DS_{rel} obtained on the modified cotton gauze without obtaining the unacceptable surface gelling was 0.85, achieved with 25% (w/v) monochloroacetic acid, 45% (w/v) sodium hydroxide and a 4 h reaction period (Table 1). When

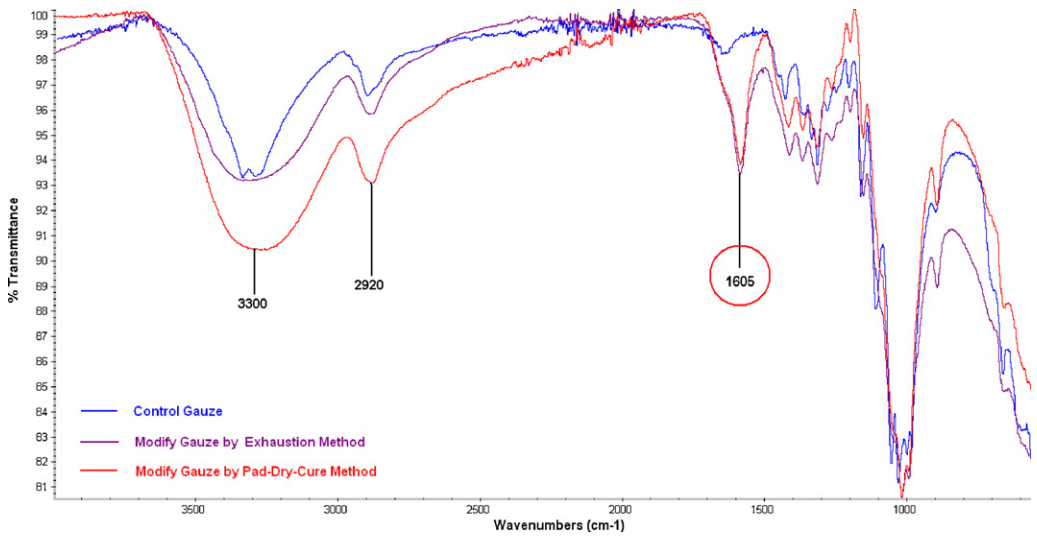

Fig. 1. Representative FT-IR spectrum of the unmodified cotton gauze and carboxymethylated cotton gauze modified by the exhaustion or the pad-dry-cure method.

using the pad-dry-cure method, the corresponding maximum DS_{rel} obtained was about 1, obtained using 25% (w/v) monochloroacetic acid, 35% (w/v) sodium hydroxide and a 3 min curing time at 120 °C (Table 2).

Carboxymethylated-modified cotton gauzes with DS_{rel} values of 0.43, 0.58, 0.68 and 0.84 obtained by the exhaustion method, and those with DS_{rel} values at 0.24, 0.46, 0.74 and 1.00 from the pad-dry-cure method, were chosen for testing the absorption and retention of silver nitrate and chitosan solutions, the bursting strength, water absorption and whiteness. However, for evaluating any effect upon the whole blood clotting rate and antibacterial activities only the unmodified (control) cotton gauze and then those with a DS_{rel} of 0.43 and 0.84 from the exhaustion method, and DS_{rel} values of 0.46 and 1.00 from the pad-dry-cure method were utilized.

3.3. Retention of the 0.5% (w/v) silver nitrate solution

Using the modified dunk-and-drain test with a 0.5% (w/v) silver nitrate solution, the amount of silver nitrate solution retained (as a percentage by weight) in each tested sample of unmodified and modified cotton gauze samples after drying for up to 2 h are reported in Table 3. At zero time in samples produced by both the exhaustion and the pad-dry-cure methods, the higher the DS_{rel} of the modified (carboxymethylated) cotton gauze the higher the amount of silver nitrate solution was retained. As the drying time increased to 2 h, the amount of silver nitrate solution retained on the carboxymethylated cotton gauze samples decreased with time, which corresponded to the drying time. However, the carboxymethylated cotton gauze had the capacity to hold a larger amount of silver nitrate solution for a much longer period of time than the unmodified cotton gauze. Thus, the use of partially carboxymethylated cotton gauze would potentially be able to protect the wound from subsequent or existent infections for a much longer period of time than that of unmodified cotton gauze.

3.4. Retention of the 0.5% (w/v) chitosan solution

Using the modified dunk-and-drain test with a 0.5% (w/v) chitosan solution, the amount of absorbed chitosan solution and the amount retained solution (as a percentage by weight) on the unmodified and carboxymethylated cotton gauze samples after drying for up to 5 h are reported in Fig. 2 for samples obtained by the exhaustion and the pad-dry-cure methods. At a drying time of zero (i.e. absorption level), for both the exhaustion and the pad-dry-cure methods, the higher the DS_{rel} of the gauze sample the higher the amount of chitosan solution was absorbed. This may be because of the electrostatic interaction between the negative charges of carboxyl ions (COO^-) adhering to the positive charges of amine groups ($-NH_3^+$) of chitosan, at least at acidic pH values (pH <5.5) such as was used in this assay. As the drying time increased up to 5 h, the amount of chitosan solution retained in the carboxymethylated cotton gauzes decreased, corresponding to the drying time, and this appeared to be independent the method of treatment (exhaustion versus pad-cure-dry method).

However, carboxymethylated cotton gauzes had a much larger capacity to absorb and retain the chitosan solution than those of silver nitrate. This was because the silver nitrate solution being in 85% (v/v) ethanol compared to the 98% (v/v) water of chitosan, is likely to have evaporated much quicker for the same energy input. Thus, the chitosan solution was retained on the carboxymethylated cotton gauze for longer. The use of partially carboxymethylated cotton gauze coated with chitosan or silver nitrate would provide some antimicrobial activity that could be an advantage of carboxymethylated cotton gauze.

3.5. Whole blood clotting time (WBCT)

The histogram in Fig. 3 demonstrates the ability of each tested carboxymethylated-modified ground cotton gauze of a different DS_{rel} to alter the rate of whole blood coagulation compared with those of the control (unmodified ground gauze) and blank (no gauze, just fresh blood only). The results revealed that when fresh blood was mixed with the ground carboxymethylation modified cotton gauze obtained by the exhaustion and the pad-dry-cure methods, with a DS_{rel} of 0.43 and 0.46, respectively, the WBCT was reduced to 10.6 and 9.8 min, respectively, which is less than the 11.8 min observed with the control gauze and 16.6 for fresh blood. However, when the DS_{rel} was increased to 0.84 and 1.0 for the exhaustion and the pad-dry-cure samples, respectively, the WBCT was increased to 13.4 and 11.6 min, respectively. Thus, the effects of carboxymethylated modification of cotton gauze upon the WBCT were essentially independent of the method of treatment (exhaustion versus pad-dry-cure method), but potentially dependent upon the DS_{rel} level. Potentially then only a certain density of negatively charged or carboxyl groups on the modified cotton gauze is optimal for promoting blood coagulation. When above this optimal level, the way to clot the blood may be different from than that with a lower carboxyl group density on the unmodified or weakly

Table 3
Silver nitrate solution retention on untreated and modified (carboxymethylated) cotton gauzes obtained by either the (A) exhaustion method or (B) pad-dry-cure method with the indicated DS_{rel} value.

DS_{rel}	Silver nitrate solution retention (% by weight) at different drying times			
	0 h	½ h	1 h	2 h
(A) Exhaustion				
(Control) 0.00	473.6 ± 10.4	61.9 ± 7.5	5.7 ± 1.3	1.5 ± 0.5
0.43	478.6 ± 11.5	110.0 ± 6.9	13.6 ± 2.6	6.7 ± 2.0
0.56	507.5 ± 10.7	118.5 ± 5.7	15.2 ± 2.7	8.2 ± 1.9
0.68	536.4 ± 12.6	126.6 ± 6.8	16.8 ± 2.9	9.5 ± 2.2
0.84	539.9 ± 13.9	130.1 ± 7.5	17.1 ± 2.8	9.8 ± 2.4
(B) Pad-dry-cure				
(Control) 0.00	464.0 ± 09.5	60.9 ± 6.9	4.9 ± 1.6	1.1 ± 0.1
0.24	479.0 ± 10.0	86.6 ± 6.0	7.0 ± 1.4	4.9 ± 0.8
0.46	527.7 ± 10.1	108.8 ± 8.5	13.3 ± 3.1	6.2 ± 1.3
0.74	531.9 ± 09.8	115.0 ± 8.2	17.7 ± 3.3	10.0 ± 1.6
1.00	583.8 ± 11.4	132.0 ± 10.1	22.8 ± 3.2	12.9 ± 3.0

modified (low DS_{rel}) cotton gauze. It is important to note, however, most people believe that normally cotton cellulose does not have ability to reduce the blood clotting time at all, yet here the unmodified (control) cotton gauze used in this study had the ability to reduce the WBCT. Whether this is due to the treatment process used in the company which supplied the origin cotton gauze for us to study is unclear and clearly if so it may have masked any effect on the WBCT induced by the carboxymethylated-modified cotton gauze. Thus, further study is required to compare the WBCT of other commercial cotton gauzes with and without modification by partial carboxymethylation.

3.6. Antibacterial properties of the modified cotton gauzes

Carboxymethylated cotton gauzes modified by the exhaustion method to a DS_{rel} at 0.43 and 0.84 showed 99.5% and 97.3% reduction of *S. aureus*, respectively, and a 100% reduction of *E. coli* in both cases (Table 4). In some contrast, the carboxymethylated cotton

Table 4
Antibacterial properties of the unmodified (control) and carboxymethylated-modified (CM) cotton gauzes obtained by either (A) the exhaustion method or (B) the pad-dry-cure method with the indicated DS_{rel} value.

Type of cotton gauze	Type of bacteria	% reduction of bacteria
(A) Exhaustion method		
Control	*Staphylococcus aureus*	0.0
CM-gauze (DS_{rel} = 0.43)		99.5
CM-gauze (DS_{rel} = 0.84)		97.3
Control	*Escherichia coli*	0.0
CM-gauze (DS_{rel} = 0.43)		100
CM-gauze (DS_{rel} = 0.84)		100
(B) Pad-dry-cure method		
Control	*Staphylococcus aureus*	0.0
CM-gauze (DS_{rel} = 0.46)		0.0
CM-gauze (DS_{rel} = 0.74)		20.5
Control	*Escherichia coli*	0.0
CM-gauze (DS_{rel} = 0.46)		28.6
CM-gauze (DS_{rel} = 0.74)		50.6

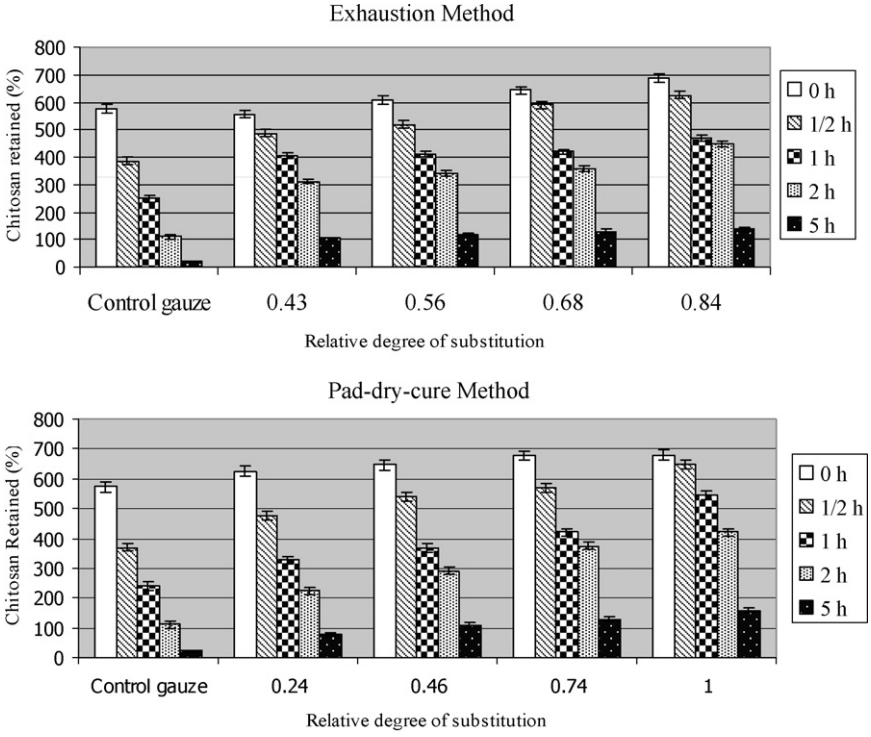

Fig. 2. Chitosan solution retained on unmodified (control) and partially carboxymethylated cotton gauzes having various DS_{rel} values after carboxymethylation via the exhaustion and pad-dry-cure methods. Data are derived from 5 repeats.

Table 5
Physical properties of the unmodified (control) and carboxymethylated cotton gauzes obtained by either the (A) exhaustion or (B) pad-dry-cure method with the indicated DS_{rel} values.

DS_{rel}	Water absorption (%)	Whiteness index	Bursting strength (kg/cm^2)
(Control) 0.00	878.9 ± 6.7	75.6 ± 1.1	0.76 ± 0.01
(A) Exhaustion method			
0.43	904.1 ± 6.1	72.2 ± 1.1	2.36 ± 0.03
0.56	945.6 ± 7.6	70.6 ± 0.8	2.56 ± 0.02
0.68	989.1 ± 7.0	70.0 ± 1.3	2.44 ± 0.02
0.84	883.4 ± 4.9	69.0 ± 1.1	2.92 ± 0.03
(B) Pad-dry-cure method			
0.24	923.9 ± 16.1	58.0 ± 0.75	1.90 ± 0.03
0.46	941.0 ± 10.9	53.7 ± 1.22	1.94 ± 0.01
0.74	955.8 ± 8.3	58.4 ± 1.18	2.08 ± 0.02
1.00	941.3 ± 6.2	53.8 ± 0.74	2.02 ± 0.04

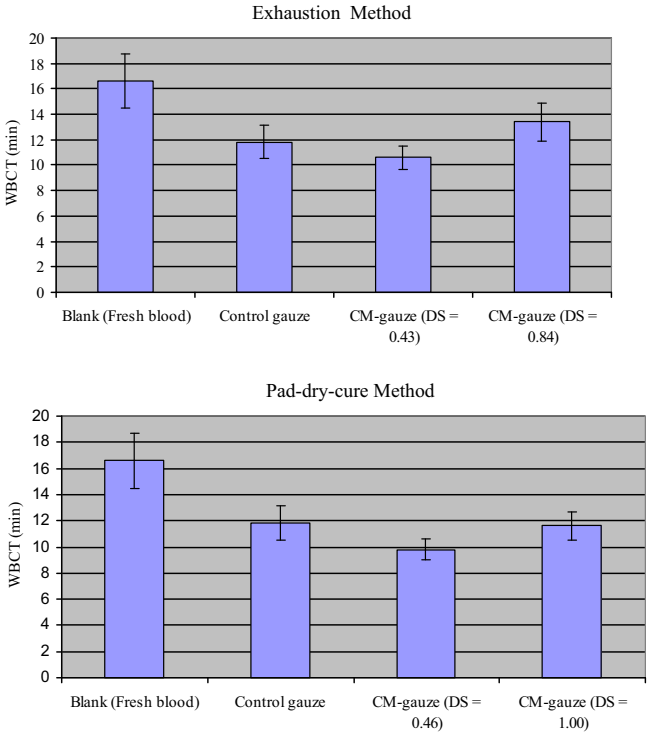

Fig. 3. WBCT without any cotton gauze (blank) or in the presence of ground unmodified (control) or partially carboxymethylated cotton gauzes (CM-) with various DS_{rel} made by the exhaustion and pad-dry-cure methods. Data are derived from three independent repeats, each with five replicates.

gauzes modified by the pad-dry-cure method showed no reduction of *S. aureus* and only a 28.6% reduction of *E. coli* at a DS_{rel} of 0.46, and at a DS_{rel} of 0.74 still showed only a 20.5% and 50.6% reduction of *S. aureus* and *E. coli*, respectively. Thus, the method used to perform the carboxymethylation of the cellulose gauze clearly affected the antibacterial efficiency of the carboxymethylated cotton gauze in addition to the DS_{rel} level. This may be because the procedure of antibacterial testing requires at least 24 h for incubation providing the very long contact time between substrate and bacteria. Therefore, such a tested sample modified both inside and on the surface of cotton gauze would show the better result of this kind of tested property.

3.7. Physical properties

The water absorption, bursting strength and whiteness index were the three physical properties investigated and the results are summarized in Table 5. The water absorption level did not significantly vary between the different carboxymethylated cotton gauze preparations with different DS_{rel} values when prepared by the pad-dry-cure method, but showed a slight but significant increase with increasing DS_{rel} values up to 0.68 when prepared by the exhaustion method, before falling to the control level at the DS_{rel} level of 0.84.

In contrast, the pad-dry-cure carboxymethylation method significantly reduced the whiteness index, and much more than that of the exhaustion method. However, this was essentially independent of the DS_{rel} and so may simply reflect the higher curing temperature used in the pad-dry-cure method (120 °C vs. 70 °C for the exhaustion method), which may then cause some decomposition of the cellulose with the associated discoloration.

Finally, the bursting strength of the carboxymethylated cotton gauzes was significantly greater than the unmodified gauze and this was greater for the exhaustion method than the pad-dry-cure method. This may be due to shrinkage, where the construction of the modified cotton gauze was tighter than that of original cotton gauze and so has a better bursting strength than that of the loose cotton gauze. The carboxymethylated cotton gauze obtained from the exhaustion method had a higher bursting strength than that obtained from the pad-dry-cure method was because of the immersion time in the exhaustion carboxymethylation much longer time than that of pad-dry-cure carboxymethylation, allowing a greater change in the cross-section of the cellulosic fibers to become rougher and experience more shrinkage.

4. Conclusions

Cotton gauze was modified by partial carboxymethylation to various degrees by both the exhaustion and the pad-dry-cure preparatory methods. Both methods could be adjusted to derive cotton gauze with different DS_{rel} values, depending on the three reaction parameters of the reaction and cure time and the concentration of the monochloroacetic acid and sodium hydroxide solutions. Under the conditions tested here it was found that cotton gauzes carboxymethylated to a DS_{rel} value of more than 1 were not acceptable, showing signs of becoming hard or gelling. The maximum DS_{rel} of the carboxymethylated cotton gauze obtained by the exhaustion method without surface gelling was 0.85, from treatment with 25% (w/v) monochloroacetic acid, 45% (w/v) sodium hydroxide and a 4 h reaction time. The corresponding maximum DS_{rel} on the carboxymethylated cotton gauze obtained by the pad-dry-cure method was about 1, derived from a 25% (w/v) monochloroacetic acid, 35% (w/v) sodium hydroxide solution and a 3 min curing time at 120 °C. The tested properties of the cotton gauzes with different carboxymethylated DS_{rel} values revealed that a higher DS_{rel} value led to a better absorption level and subsequent retention time of chitosan and silver nitrate solutions. The WBCT was significantly reduced by the unmodified cotton gauze, whilst this was only slightly further reduced by the carboxymethylated cotton gauze with a DS_{rel} of 0.43 and 0.46 derived from the

exhaustion and the pad-dry-cure methods, respectively, and increased (compared to unmodified gauze) at higher DS_{rel} values for both production methods. In terms of antibacterial activity, only carboxymethylated gauzes with DS_{rel} values of 0.43 and 0.84 from the exhaustion method showed any significant antibacterial activity, whilst the carboxymethylated cotton gauze at similar DS_{rel} values obtained from the pad-dry-cure method, and the unmodified gauze could not inhibit the test bacteria effectively. This may be because the immersion and reaction time of the exhaustion method were much longer than that for the pad-dry-cure method, and so allowing the carboxymethylation reaction to occur both inside and on the surface of the cotton gauze. The bursting strength of the different cotton gauzes was the greatest with the carboxymethylated cotton gauze treated by the exhaustion method compared to that of the pad-dry-cure method. Again this was likely to be because of the much longer immersion time in the reagents in the exhaustion method compared to that of the pad-dry-cure method, and so could provide more shrinkage on the cotton gauze and make the structure of the cotton gauze tighter.

The pad-dry-cure method, however, was observed to significantly reduce the whiteness index, and much more than that of the exhaustion method, but this was independent of the DS_{rel} values. This may hence simply reflect the higher curing temperature of the pad-dry-cure method (120 °C vs. 70 °C) leading to decomposition and charring of some of the cellulose.

Finally, the water absorption of the carboxymethylated cotton gauzes increased slightly with increasing DS_{rel} values when prepared by the pad-dry-cure method, but this was more marked when prepared by the exhaustion method up to a DS_{rel} value of 0.68, and declined thereafter.

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