Moist Wound Dressing Fabrications: Carboxymethylation of Antibacterial Cotton Gauze

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ABSTRACT

Cotton gauze wound dressing materials were carboxymethylated in order to maintain moist conditions by increasing the carboxyl contents on their cellulose moieties. Also, to improve their antibacterial efficacy, two synergistic drugs were treated after carboxymethylation.

Carboxymethylation of the fabrics was carried out using different concentrations of sodium hydroxide/mono chloro acetic acid (NaOH/MCA) to increase the carboxyl contents (85, 173, 246 mmol/100g). To acquire moist conditions of the fabrics, the carboxyl contents of the gelling samples were made in Ca/Na forms (Na ions was replaced by Ca ions) in three different forms of degree of neutralization (10/90, 20/80, and 30/70). The first non-gelling sample (85 mmol/100g) was left in its Na form to differentiate its chemical and biological properties from the other two samples. In order to fabricate the carboxymethylated (CM) cotton with antibacterial drugs, all the samples were padded in different concentrations (100, 500 and 1000 mg/L) of two synergistic drugs: ofloxacin and ornidazole. These samples were considered as carboxymethylated antimicrobial dressings (CM-AMD). The impact of antibacterial drug content on swelling, gelling, and shrinkage was determined along with the antibacterial activity. Both qualitative and quantitative antibacterial activity was measured for CM-AMD samples.

Among the three samples, the first sample (85 mmol/100g) showed no gelling in phosphate buffered saline (PBS), whereas the other two samples (173 and 246 mmol/100g) showed gelling visually. In the present research, it was observed that swelling and shrinkage slightly decreased by increasing the concentrations of antibacterial drugs; and increased by increasing the carboxyl content. The antibacterial activity of CM-AMD when tested qualitatively showed that, the inhibition clear zone (ICZ) increased by increasing drug concentrations and carboxyl contents; but decreased by increasing the degree of neutralization. Quantitative bacterial reduction testing showed 100 % reduction of the test organisms (*Staphylococcus aureus* and *Peptostreptococcus* sp)

The study concludes that carboxymethylated cotton gauzy fabrics containing more carboxyl contents (≥ 246 mmol/100g) with less degree of neutralization (10 %) treated with synergistic antibacterial drugs could be suitable for fabricating antibacterial wound dressing materials.

Keywords: Carboxyl contents, swelling, gelling, shrinkage, antibacterial activity, inhibition clear zone

INTRODUCTION

Infection and bacterial colonization remain very important factors in delayed wound healing [1]. Since the wide spread use of systemic and topical antibiotics has resulted in increasing numbers of resistant bacterial strains (methicillin - resistant *Staphylococcus aureus* and vancomycin resistant *Enterococcus faecalis* and *Pseudomonas aeruginosa*) ,it has been suggested that the judicious use of antimicrobial dressings, notably those containing certain antiseptics, can be important in infection control and in promoting healing [2]. According to Ovington, moist wound healing is to keep the exposed wound tissue at optimum hydration that is, wound tissues should be physiologically moist, not wet but not dry [3]. Accordingly, moist wound healing dressings can be classified into three types: absorbent dressings that have a high capacity for capturing and holding fluids, moist maintaining dressings that can keep the level of natural moisture of the newly forming tissues without active absorption, and moisture donating dressings [4].

Campbell described that the newer dressings are designed to create a moist wound healing environment which allows the wound fluids to remain in contact with wound [5]. Jones and Harding, proposed different benefits of moist wound healings

including prevention of the formation of a scab, which can trap white blood cells which prevents them from participating in their important wound healing functions; reduction of the pH of the environment, thus adversely affecting bacteria; prevention of bacterial strike through from outside the wound to the wound surface; more rapid epithelialisation; and fact that the moist environment favors colonization of bacteria but not infection. Although a moist wound favors colonization of bacteria and increases bacterial numbers, infection rates are not increased in most studies probably due to increased white blood cell function [6].

Carboxymethylated cotton was found to be a cheaper competitive for alginate fibers in the production of absorbent wound dressings [7]. Attwood described that both dressings are able to absorb exudates from chronic wounds and maintain a moist environment that simulate platelet activation and blood coagulation [8]. According to Parikh *et al*., blood coagulation occurs due to the release of Ca ions in the wound environment by the ion exchange process [7]. Abo-Shosho *et al*.in their study replaced Na ions with Ca ions to understand the exudate absorption during the early phase of contact with the wound. The main disadvantage of these dressings is their non-resistance to bacterial infection [4]. Tereschenko and Shamolina expressed that a dressing saturated with wound discharge is a favorable medium for the growth of pathogenic bacteria. This results in increasing the levels of bacteria and bacterial products [9]. According to Yudanova and Reshetov, these biochemical imbalances commonly found in chronic and non-healing wounds will results in stalling conditions [10]. Abo-Shosho *et al* treated tetracycline hydrate and gentamicin in carboxymethylated cotton gauze to study their antibacterial activity against significant clinical pathogens, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [4].

Based on these factors, in the present study clinically significant cotton gauze was initially carboxymethylated (CM) followed by treatment with antibacterial agents to achieve functional antimicrobial dressings (AMD). Carboxymethylated cotton gauze fabrics of different contents of carboxyl groups in the form of variable degrees of neutralization by Ca/Na cations was treated with selected synergistic drugs. Two different groups of synergistic antimicrobial drugs, a fluoroquinolone (ofloxacin) and a nitroimidazole (ornidazole), were selected for antimicrobial finishing. The drugs were selected based on the factors described by Saginur *et al*.[11] and Boeckh *et al*. [12]. Saginur *et al*. reported that the accepted clinical practice to treat biofilm-associated infections was the use of combination therapy in which two or more antimicrobials are blended at different combinations to achieve a broader spectrum of activity at a lower concentration resulting in more effective therapy and decreased resistance [11]. Similarly, Boeckh *et al* suggested expansion of the antibacterial spectrum by combining quinolones with other antibacterial agents for preventing biofilm formation [12]. In our previous study, synergistic drug combinations of norfloxacin and metronidazole coated ureteral stents were found to prevent biofilm forming organisms like *Staphylococcus epidermidis* and *Escherichia coli* [13]. By measuring the carboxyl content, swelling, and shrinkage the CM-AMD was characterized in the present study. The effect of the increase in carboxyl contents with variable degrees of neutralization by Ca/Na cations was analysed for changes in physical, chemical, and biological properties. Physical and chemical properties are carboxyl contents, swelling, and shrinkage behavior of CM and CM-AMD cotton gauze fabrics. Biological properties of CM-AMD were evaluated using qualitative and quantitative antibacterial assays. Antibacterial activity towards *Staphylococcus aureus* (aerobic/facultative anaerobic) and *Peptostreptococcus* sp (anaerobic) was selected based on their clinical significances in diabetic foot wounds. A HET-CAM test was performed to identify the inflammatory reactions of CM-AMD after implantation on the chorioallantoic membrane of embryonated chick eggs.

MATERIALS AND METHODS

In the present research, carboxymethylation, qualitative agar diffusion testing and quantitative bacterial reduction testing were carried out in the Research laboratory of the Microbiology Department at the CMS College of Science and Commerce in Coimbatore, India. Carboxyl content, swelling, and shrinkage behavior of CM cotton was tested in the Textile Research laboratory, NIFT-TEA College of Textiles, Tirupur, India. Treatment of CM cotton with antibacterial drugs using a padding mangle was performed in the Research laboratory of the Microbiology Department at PSG College of Arts and Science, Coimbatore, India. The entire research work was carried out from October, 2011 to June 2012

Materials

Cotton Gauze - Mill scoured and bleached 100% cotton gauze fabric (55 g/m^2), Chemicals (HiMedia and Qualigens, India) - monochloroacetic acid (MCA) and sodium hydroxide (reagent grades), poly

vinyl pyrrolidone (PVP), Antibacterial drugs – Ofloxacin and Ornidazole (Ranbaxy, India)

Test Bacterium

Clinical isolates, *Staphylococcus aureus* and *Peptostreptococcus* sp. was isolated from the wound dressing materials of diabetic foot patients. All the strains were cultured to late logarithmic growth phase on blood agar plates at 37°C for 18 h before testing. Bacterial cells grown to 3.0 X 10^4CFU/ml were used for testing.

Methods

Carboxymethylation (Heydarzadeh et al., 2009)

Carboxymethylated cotton gauze fabric was prepared using a standard exhaustion method [14]. Alkali cellulose was prepared by impregnating the fabric in 5% sodium hydroxide solution in 95/5 ethanol/water for 15 minutes at room temperature, then padded to a wet pick-up of 100% and air dried. The fabric was lightly rolled and introduced into a 1L stoppered glass bottle containing a mixture of NaOH/monochloroacetic acid (Na salt), at a molar ratio of 2/1, in 50/50 ethanol water using a material to liquor ratio of 10. The bottle was put in a thermostated shaking water bath for 5 hours at 50 °C. The fabric was then taken out of the bottle, squeezed and the excess alkali was removed by washing with 0.1N acetic acid (in 80/20 ethanol/water) until neutralization with phenolphthalein.

Ca/Na CM Cotton (Parikh et al., 2003)

In order to partially replace sodium ions (Na^+) by calcium ions (Ca^{++}) in CM cotton, the carboxyl groups were partially transformed in acid form by reaction with the equivalent amount of HCl in 80/20 ethanol water (to get 10, 20, and 30% degree of hydrogen form) for 24 hours at room temperature, followed by thorough washing with 80/20 ethanol/water. The fabric was then reacted with 80/20 ethanol water solution containing $CaCl_2$ in amounts equivalent to 10, 20, and 30% of the carboxyl groups for 24 hours at room temperature, followed by thorough washing with 80/20 ethanol/water.

Ofloxacin and Ornidazole treatment (Abo-Shosha et al., 2009 method)

Polyvinylpyrrolidone (PVP) was utilized to facilitate the solubility of antibacterial drugs. 5 g of PVP was dissolved in 100mL of 80/20 ethanol/water and in this solution ofloxacin and ornidazole was dissolved at three different concentrations (100 mg/L, 500 mg/L, 1000 mg/L). Ca/Na CM cotton fabrics were padded in any of these solutions at a wet pick-up of 100%. Padding was carried out in a pneumatic mangle at a pressure of 3 psi to obtain a pick-up of 100 % on weight of fabric. Drying and curing were carried out at 80 °C for 5 min and 150 °C for 3 min respectively.

Determination of Carboxyl Content - ASTM D 1926 - 00(2011) Standard Test Method

During the exhaustion method, test fabric exposed to three different concentrations of NaOH/MCA [(mol/L) / (mol/L] was tested to determine its carboxyl contents using a standard ASTM D1926 – 00(2011) method [15].

Swelling ability of CM cotton (Ehrhardt et al., 2007)

Swelling (water-uptaking ability of CM cotton) was measured by impregnating the fabric in phosphate buffered saline (PBS, pH 7.4) at 37 °C for 24 hours [16]. After incubation the fabric was removed, wiped gently with a filter paper and weighed.

Swelling percentage (%) was calculated using the formula under given.

$$\text{Swelling} = 100 \times \frac{(m_1 - m_0)}{m_0}\,\%$$

where, m1 and m0 are the weight of sample after and before impregnation.

After removing the sample from PBS, simultaneously, a gelling sample was assessed visually. Gelling was detected by identifying a swelled-semi-transparent smooth surface on the fabric.

Evaluation of Shrinkage (ASTM D 1905 standard test method)

Shrinkage was evaluated by soaking a 10 x 10 cm fabric sample in phosphate buffered saline (PBS, pH 7.4) at 37 °C for 24 hours [17]. Change in area or Shrinkage was calculated using the following formula.

$$\text{Shrinkage} = 100 \times \frac{(a_1 - a_0)}{a_0}\,\%$$

where, a1 and a0 are the areas before and after impregnation.

Antibacterial Activity

Agar diffusion test (AATCC Test Method 147 - 1998)

Antibacterial activity towards *S. aureus* and *Peptostreptococcus* sp was evaluated by the qualitative agar diffusion test [18]. In this method the pre-measured size (2.5 cm^2) of the test materials

were tested from each preparation (10%, 20% and 30% degree of neutralization). Test materials were placed on the surface of Mueller-Hinton agar plate which had previously been seeded with an overnight broth culture of the test organisms and incubated at 37°C for 24 to 48 hours. Antibacterial activity was expressed as the diameter of the zone of inhibition or inhibition clear zone (ICZ).

Bacterial reduction test (AATCC test method 100 - 2004)

The CM-AMD cotton with selected degree of neutralization showing more ICZ against test organisms in qualitative agar diffusion test was further tested quantitatively by a standard bacterial reduction test (AATCC test method 100-2004). Briefly, 1.0 ml of 12 hours challenge bacterial inoculum was dispersed as droplets over the 3 swatches (test fabrics) using a micropipette. The swatches were inoculated in pre-sterilized 250 ml Erlenmeyer flasks. After all the samples were inoculated, the flasks were incubated at 37 ± 2 °C for 18 h before being assayed for bacterial population density. The bacterial population density was determined by extracting the bacteria from the fabric by adding 100 ml of distilled water to each flask and shaken using an orbital shaker for one minute. Then aliquots were serially diluted and pour plated to determine the bacterial density. The difference in number of viable bacteria was evaluated on the basis of the percentage reduction. Percentage reduction was calculated using the following formula.

$$R = (A-B) / A \times 100$$

Where ***R*** is percentage reduction, ***A*** is the number of bacteria in the broth inoculated with treated test sample immediately after inoculation i.e., at zero contact time and ***B*** is the number of bacteria recovered from the broth inoculated with treated test fabric sample after the desired contact period of 18 hours [19].

Hen´s Egg Test on the Chorioallantoic Membrane (Cazedey et al., 2009)

To study the allergic reactions or necrosis of drug treated carboxymethylated cotton, the gauze material was placed on the surface of chorio-allantoic membrane (CAM) of embryonated chick eggs. A standard HET-CAM protocol [20] was followed to detect the inflammatory reactions (histological evaluation). Briefly, 0.3 g of test fabric (CM treated with antibacterial drugs) was placed on chorioallantoic membrane of fertilized eggs (9 to 10 days of development) through a window made on egg shell. After nine days of incubation, the membrane was carefully dissected and stained with haematoxylin and eosin stains. The results were assessed by type of irritation or necrosis caused by the material on the membrane (lysis, bleeding or coagulation). Inflammations could be indicated by the appearance of hue colors on the CAM. 0.1 N NaOH was used as positive control (produces inflammation) and 0.9 % NaCl as negative control.

RESULTS AND DISCUSSION

Carboxymethylation of Cotton Gauze Fabric

Medically significant cotton gauze fabric used as wound dressing material was carboxymethylated using three different concentrations of NaOH/mono chloro acetic acid to increase the carboxyl contents in the material. Increase in carboxyl contents were attained on cotton gauze fabric by changing NaOH/Mono chloro acetic acid concentrations in the exhaustion bath. Three different levels of carboxyl contents (85, 173 and 246 mmol/100g) was obtained on the fabric. Swelling and gelling properties of each level of carboxyl content fabric is shown in *Table I.* Cotton gauze sample containing the carboxyl content of 85 mmol/100 g showed no gelling in phosphate buffered saline. The sample having more carboxyl contents (173 and 246 mmol/100g) showed good gelling properties.

TABLE I. Carboxyl content, swelling and gelling behavior of cm cotton fabric padded with different naoh/mca concentrations.

Concentration of NaOH/MCA (2:1) (mol/L)	Carboxyl content of fabric (mmol/100 g)	Swelling (%)	Gelling
4/2	85	410	No
6/3	173	805	Yes
8/4	246	925	Yes

Swelling and gelling is conducted in PBS at 37°C for 24 hours. Cotton gauze fabric was padded with different concentrations of NaOH/MCA (mol/L) at 2;1 ratio

Ca/Na Cm Cotton – Formation of Ca/Na Carboxylate

In order to partially replace Na^+ by Ca^+ in CMC cotton, the carboxyl groups were partially transformed in acid form by reaction with the equivalent amount of HCl in 80/20 ethanol water (to get 10, 20, and 30% degree of hydrogen form). On this regard, the carboxyl groups of the first sample (fabric containing 85 mmol/100g) were left in their Na salt, whereas the other two gelling samples (173 and 246 mmol/100g) were made in Ca/Na forms in three different degrees of neutralization, viz. 10/90, 20/80, and 30/70. The Na form (sodium carboxylate) in the CM cotton absorbs exudates during the early phase of contact with the wound. Since the Ca form limits this absorption, Na was replaced with Ca

cations, so that the wound not get desiccated and kept always moist. According to Abo-Shosho *et al*., (2009) Ca is replaced by sodium according to the following equilibrium [4]:

$$(Cell-COO)_2Ca + 2Na^{+}(\text{in wound fluids}) \rightleftharpoons 2Cell-COONa + Ca^{++}$$

According to Parikh, *et al*., the replaced Ca^{++} cations get precipitated by coagulating blood, so that equilibrium of the above reaction is shifted again towards the formation of more sodium carboxylate. Thus formed sodium carboxylate is necessary to absorb the newly produced exudates, which helps in controlling the moist environment at optimal level. Parikh, *et al*., also reported that gelling is needed for that part of a dressing in contact with the wound in order to reduce friction, so that the newly formed tissues are not adhered to the dressing [7].

Abo-Shosho *et al*. designed the fabric to make multilayer, in which the inner layer was designed in such a way to have contact with skin that manifests high ability to absorb exudates (swelling) and gelling behavior (without dissolution in the wound medium). The outer layer should also showed reasonable swelling to absorb any leaking exudates, without the necessity to manifest gelling. With the similar expectations, in the present study CM cotton gauze fabrics each containing different levels of carboxyl contents were fabricated with two antibacterial drugs (ofloxacin and ornidazole) to resist local and invading infections that is to prevent the adherence of bacterial pathogens in both inner and outer layers. In the next sections Ca/Na containing CM cotton fabrics treated with antibacterial drugs (CM-AMD) were characterized by investigating their swelling and gelling, shrinkage, antibacterial activity and biocompatibility.

TABLE II. Swelling and gelling behavior of CM-AMD fabrics containing different concentrations of ofloxacin and ornidazole.

Concentration of drugs in CM-AMD samples (mg/L)	Carboxyl content (mmol/100 g)						
	85	**173**			**246**		
	Degree of neutralization with Ca cations (%)						
	0	10	20	30	10	20	30
	Swelling (%)	Swelling (%)	Swelling (%)	Swelling (%)	Swelling (%)	Swelling (%)	Swelling (%)
Untreated CM	405	802	721	636	**930**	845	725
100	401	795	718	624	921	842	706
500	398	786	709	620	895	830	698
1000	**385**	780	705	614	890	805	682

Swelling and gelling is conducted in PBS at 37°C for 24 hours.
85 mmol/100 g fabric samples: no gelling was observed
173 and 246 mmol/ 100 g fabric samples: gelling was observed
CM cotton was padded with synergistic drugs, ofloxacin and ornidazole for the fabrication of CM-AMD fabrics

TABLE III. Shrinkage behavior of CM-AMD fabrics containing different concentrations of ofloxacin and ornidazole.

Concentration of drugs in CM-AMD samples (mg/L)	Carboxyl content (mmol/100 g)						
	85	**173**			**246**		
	Degree of neutralization with Ca cations (%)						
	0	10	20	30	10	20	30
	Shrinkage (%)	Shrinkage (%)	Shrinkage (%)	Shrinkage (%)	Shrinkage (%)	Shrinkage (%)	Shrinkage (%)
Untreated CM	20	52	45	36	55	49	48
100	18	48	39	31	48	44	45
500	12	44	32	29	44	37	40
1000	8	39	29	22	38	31	32

Shrinkage in PBS at 37°C
CM cotton was padded with synergistic drugs, ofloxacin and ornidazole for the fabrication of CM-AMD fabrics

Swelling and Gelling of CM cotton and CM-AMD

Effect of treatment with three different concentrations of ofloxacin and ornidazole on the swelling and gelling of CM-AMD of different carboxyl (Na salt) and Ca contents is shown in *Table II*. The analysis showed that, the untreated samples (CM cotton) had slightly higher swelling than the drug treated fabrics (CM-AMD). Also it was observed that when the drug concentration was increased the extent of swelling was slightly decreased. Another significant factor of carboxyl content also influenced the swelling properties in the

fabrics. It was found that the higher the carboxyl content (10 % degree of neutralization) is the higher the fabric swelling, and the higher the Ca content (20 % and 30 % degree of neutralization) is the lower the fabric swelling. However, the drug treated samples showed a wide margin of swelling ranging from 385 % to 930 % could give rise to varieties of choice in designing CM-AMD. Similar findings were reported by Abo-Shosho *et al*., (2009) when they used tetracycline hydrate and gentamicin sulphate treated fabrics with carboxyl contents of 72, 170 and 220 meq/100g.

Shrinkage of CM Cotton and CM-AMD

Shrinkage percentage of CM and CM-AMD fabric samples are shown in *Table III*. The shrinkage test was carried out with the same set of conditions provided for fabric swelling. It can be seen from *Table II* that, shrinkage also followed the same direction of swelling for treated and untreated samples. Ehrhardt *et al* reported that, swelling results in increasing the fibers and yarn diameter, this in turn brings about a contraction in their lengths and hence leading to fabric shrinkage [16]. In simple terms, when fabric swells more, the extent of shrinkage will be large. Hence it was recommended that the dimensions of a dressing should be 5 cm larger than those of a wound.

Antibacterial Activity of CM-AMD Fabrics

Agar Diffusion Test

Antibacterial activity of CM-AMD was expressed as inhibition clear zone (ICZ) against the test organisms *Staphylococcus aureus* and *Peptostreptococcus* sp. Different significant factors like antibacterial drug, carboxyl and Ca contents highly influenced the inhibition of the growth of bacteria around CM-AMD samples. Untreated fabrics failed to restrict the growth of bacteria around the CM cotton samples. From *Table IV* it is evident that the ICZ was increased after increasing the drug concentration and carboxyl content (85, 173 and 246 mmol/100g). Also it was observed that ICZ was decreased by increasing Ca content (20 % and 30 % degree of neutralization).

TABLE IV. Qualitative test - antibacterial activity of CM-AMD treated with different concentrations.

Concn. of drugs in CM-AMD samples (mg/L)	Carboxyl content (mmol/100 g)													
	85		**173**						**246**					
	Degree of neutralization with Ca cations (%)													
	0		10		20		30		10		20		30	
	Inhibition Clear Zone (ICZ in mm)													
	SA	PS	SA	PS	SA	PS	SA	PS	SA	PS	SA	PS	SA	PS
Untreated CM	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	10.3	9.3	11	10.6	11.9	10.3	11.9	9.9	15.6	13.6	15.9	12.3	14.3	12.6
500	12.3	11.6	15.3	12.3	13.3	12.3	14.6	11.6	19.3	17.3	18.3	15.3	17.3	15.9
1000	18.6	16.3	19.3	18.9	21.0	17.9	19.6	17..3	24.6	21.3	21.6	19.9	18.9	18.6

SA – *Staphylococcus aureus*, PS – *Peptostreptococcus* sp (tested under strict anaerobic conditions)
Tested in triplicates, Mean values are tabulated
CM-AMD fabrics containing the carboxyl contents (246 mmol/100g) with 10 % degree of neutralization shows more ICZ for test organisms

The reason was reported by Abo-Shosho *et al*. They reported that, increasing the extent of cross linking of the fabric structure by the divalent Ca cations (20 % and 30 %) would restrict the diffusion of antibacterial drugs from CM-AMD fabrics. This was evident from the inhibitory zones measured in millimetre presented in *Table V*. Maximum inhibitory zone (24.6 mm and 21.3 mm) was obtained for CM-AMD fabric samples (10% degree of neutralization) containing 246 mmol/100g treated at a concentration of 1000 mg/L against the test organisms. Whereas for the similar carboxyl levels of CM-AMD fabric samples (20 % and 30 % degree of neutralization) treated at similar concentration (1000 mg/L) showed inhibitory zones (*Table IV*) less than that of the former sample (10 % degree of neutralization). *Figure 1* shows the ICZ of CM-AMD fabrics (246 mmol/100 g) with 10 %, 20 % and 30 % of Ca content against the test organisms, *Staphylococcus aureus* (*Figure-1a*) and *Peptostreptococcus* sp (*Figure-1b*).

1(a) S. *aureus* **1(b) *Peptostreptococcus* sp**

FIGURE 1. Qualitative antibacterial activity of test bacteria. uc – uncoated or untreated cotton. 10%, 20 %, 30% -CM-AMD (246 mmol/100g) with different degree of neutralization. *Peptostreptococcus* sp was incubated under strict anaerobic conditions (Mc Intosh jar)

Bacterial Reduction Test

The CM-AMD cotton samples treated at a concentration of 1000 mg/L with 246 mmol/100g carboxyl contents, with 10 % degree of neutralization showing maximum ICZ against test organisms in qualitative agar diffusion test were tested quantitatively by a standard bacterial reduction test (AATCC test method 100-1999). The antibacterial activity showed good bacterial reduction percentage of 100 % against both the test organisms. In *Table V* the bacterial reduction percentage for each test bacteria are presented after calculating the CFU at 0^{th} hour and 18^{th} hour. Quantitative bacterial reduction testing showed supportive test results to the former agar diffusion test. Both the tests influenced the factors like increase in the drug concentration, increase in the carboxyl content and decrease in the Ca cations increased the antibacterial activity of CM-AMD cotton fabrics. The antibacterial activity of the drugs used to treat the CM cotton has a characteristic feature of synergism. Antibacterial efficacy of the drugs was clearly understood from their similar mode of action. In the present research the drug combinations (Ofloxacin-fluoroquinolone and ornidazole-nitroimidazole) selected were mainly based on their mode of action on DNA of prokaryotic organisms. According to Chu and Fernandes, the fluoroquinolones are strong bactericidal agents, effective against a broad spectrum of gram-positive and gram-negative bacteria [21]. Fluoroquinolones target the bacterial type II A DNA topoisomerases DNA gyrase and DNA topoisomerase IV. These hetero-tetrameric enzymes manipulate DNA topology by introduction of transient double-stranded breaks in bound DNA (G-segment) through which a second DNA fragment (T-segment) may be passed [22]. Binding, by intercalation [23], of quinolone antibiotics to the complex of enzyme and the cut G-segment stabilizes this so-called cleavage complex, leading to accumulation, and eventual release, of double-stranded DNA breaks that are ultimately lethal to the cell [24].

Significant progress in the study of nitroimidazoles came in 1977, when it was found that chemically reduced metronidazole caused destabilisation of the DNA helix and strand breakage in vitro thus enabling model systems to be used [25]. Although protein has been suggested as a possible site of action of nitroimidazole drugs on the basis that about 30 % of these drugs binds to intracellular components, no evidence has been published to suggest that any important protein system is inhibited. Instead, evidence based on the suggestion of Ings *et al*. that DNA is the major site of action of nitroimidazoles [26]. Ings' initial observations showed that DNA synthesis in bacteria was not only inhibited but existing DNA was also broken down.

TABLE V. Quantitative test - bacterial reduction percentage of CM-AMD fabrics (aatcc test method 100-1999).

Test organisms	Bacterial Reduction Percentage (%)		
	CM-AMD (246 mmol/100g) + 10 % Ca	CM cotton gauze	Cotton gauze (without CM)
SA	100	0	0
PS	100	0	0

CM-AMD fabrics containing the carboxyl contents (246 mmol/100g) with 10 % degree of neutralization (selected from qualitative test)
SA – *Staphylococcus aureus*, PS – *Peptostreptococcus* sp (tested under strict anaerobic conditions)

Hen´s Egg Test on the Chorioallantoic Membrane

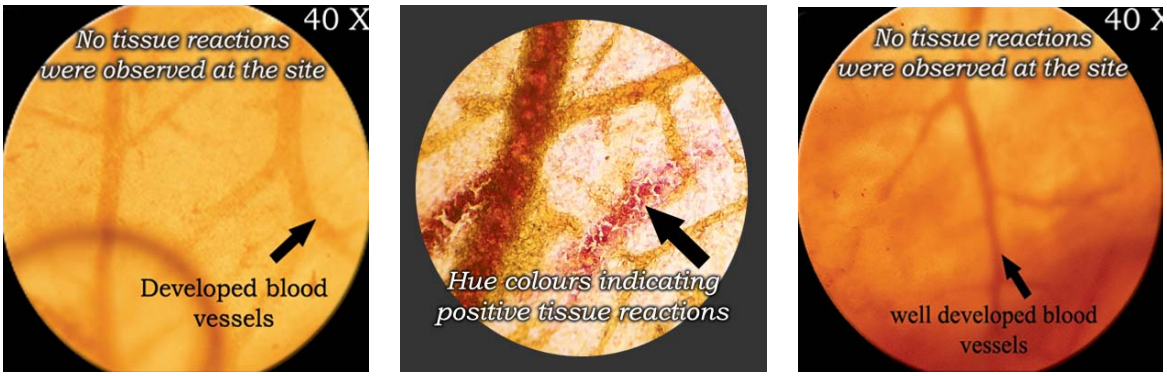
Histological Evaluation of CAM

Histological evaluation of test materials implanted CAM was observed under a bright-field microscope after staining with haematoxylin and eosin. Strong blue and hue colors of inflammatory cells due to fibrous deposition on positive control CAM samples (0.1 N NaOH) are shown in *Figure 2*. The active ingredient, hematein complexed with aluminium potassium sulphate in haematoxylin produced the strong blue color and eosin produced the shades of hue colors. Negative control (0.9 % NaCl) CAM sample showed well developed blood vessels and nucleated epithelial cells. Histological examination of the test material implanted CAM samples were compared with the interpretations of positive and negative control samples. In *Figure 2* the microscopic stained sections of CAM implanted with CM-AMD cotton samples was presented. The stained microscopic image of CM-AMD [*Figure-2(a)*] showed no tissue reactions or necrosis after comparing with the positive and negative control CAM samples [*Figure-2(b) and 2(c)*]. Even the possible mild tissue reactions like obvious edema and fibrous deposition with degenerative changes of epithelial cells was not observed for the test material.

Instead clear well developed nucleated epithelial cells with blood veins were observed indicating good biocompatible properties of CM-AMD fabrics. The CAM model is a true *in vivo* system that can be used as an intermediate step between a cell culture and a more complex mammalian model. The CAM can be used for the evaluation of both acute and chronic inflammatory responses to biomaterials (Valdes *et al*., 2001). In addition, the CAM model used in the study presented the ability to continuously visualize the implant site without having to sacrifice the test animal.

CONCLUSION

Carboxymethylation was carried out in the cotton fibers to form alkali cellulose. Due to carboxymethylation, the alkali cellulose modifies the crystalline structure of cellulose and increases the accessibility of fibres to chemicals by swelling. Hence in the present study, two different antibacterial drugs with similar mode of action were used to bind the cotton fibres. Carboxymethylated cotton gauze fabrics prepared by the exhaustion method using different concentrations of NaOH/MCA showed carboxyl contents of 85, 173 and 246 mmol/100g. The fabric containing 85 mmol/100g showed no gelling in PBS at 37 °C in contrast to those having carboxyl contents of 173 and 246 mmol/100 g. Ca/Na salts of the carboxyl groups of the gelling samples were changed in different degree of neutralization (10 %, 20 % and 30 %). The fabric containing 85 mmol/100g was left in Na form. Carboxymethylated cotton was treated with two synergistic drugs: ofloxacin and ornidazole. The drugs were selected based on their clinical significances of synergism. Both the drugs attack the DNA replicative enzymes of aerobic and anaerobic bacteria. Treated and untreated samples containing different carboxyl contents were analysed for swelling and shrinkage behavior with respect to the degree of neutralization. It was found that swelling and shrinkage slightly decreased by increasing the concentrations of antibacterial drugs, and increased by increasing the carboxyl content. CM-AMD samples of different carboxyl contents were determined for tier antibacterial activity with respect to the degree of neutralization. ICZ against both the test organisms was increased by increasing antibiotic and carboxyl contents, but decreased by increasing the degree of neutralization. From the analyses, it was suggested that the carboxymethylated cotton gauzy fabrics containing more carboxyl contents (≥ 246 mmol/100g) with less degree of neutralization (10 %) treated with synergistic antibacterial drugs could be a suitable antibacterial moist wound dress materials.



2(a): Drug treated CM Cotton 2(b): Positive control (0.1 N NaOH) 2(c): Negative control (0.9% NaCl)

FIGURE 2. Histological studies to determine tissue reactions of CM-AMD cotton. No tissue inflammations or necrosis were observed on CAM implanted with drug treated CM cotton and negative control samples. Inflammatory tissue reactions indicated by the hue colors on CAM for positive control samples. Microscopic observations: 40X magnification.

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