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Enhancement of antimicrobial efficacy of neem oil vapour treated cotton fabric by plasma pretreatment

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This paper deals with the study on the reaction between DC air plasma treated cotton cellulose and bioactive compounds present in the neem oil vapour. Untreated cotton fabric (control) and DC air plasma pretreated cotton fabric were exposed to neem oil vapour to impart antimicrobial activity, and the process parameters such as temperature and treatment time were optimised to obtain the maximum antimicrobial activity. Gas chromatography–mass spectrometry analysis was carried out to identify the bioactive compounds present in neem oil vapour. The effect of plasma treatment in modifying the fabric surface was explored by analysing the changes in the cellulose structure, crystallinity and morphology of fabric surface using attenuated total reflection–Fourier transform infrared spectroscopy (ATR-FTIR) spectra, X-ray diffractogram and scanning electron microscopy (SEM) micrographs. The reaction between cellulose and bioactive compound present in neem oil vapour was analysed using the ATR-FTIR spectra. The change in morphology after treatment with neem oil vapour was analysed using SEM micrographs.

Keywords: Cotton cellulose, DC air plasma treatment, Oxidation, Degree of crystallinity, Neem oil vapour, Tetradecanoic acid, Antimicrobial activity

Introduction

The minimal requirements for the growth and multiplication of microbes and bacteria are carbon, nitrogen and some inorganic salts. Cotton fabrics, by virtue of their characteristics and proximity to the human body, provide an excellent medium for the adherence, transfer and propagation of infection causing microbial species to proliferate [1,2,3]. Further, cotton fibres are easy targets for microbial attack because of their tendency to retain water readily, thus allowing the microbial enzymes to easily hydrolyse their polymer linkages [3,4]. Therefore, in order to avoid infection produced by microbes, the fabric must be provided with antimicrobial treatment. Antimicrobial function is a feature of medical textiles, which can be imparted to the textiles using suitable antimicrobial agents. These finishes destroy the growth of microorganisms by producing a negative effect on the vitality of the microbes and thus enhance the performance of the textiles against microbial infestation [5]. Antimicrobial agents are of natural, synthetic or semi-synthetic origin, which, at low levels of concentration, could either kill or inhibit the growth of microorganisms. Synthetic antimicrobial agents such as triclosan, metals and their salts, organometallics, phenols and quaternary ammonium compounds have been developed, and quite a few are also commercially used in textiles. The antimicrobial property of Ag nanoparticles, in particular, has been exploited in almost all industries ranging from textile to health care. However, to overcome the inherent difficulties of Ag nanoparticles, namely, stability and hydrophobicity, and also to enhance the antimicrobial activity, in recent years, alternative approaches such as binding nanoparticles onto either carbon nanotube [6] or montmorillonite [7,8] have been successfully carried out. Although the synthetic antimicrobial agents are very effective against a range of microbes and give a durable effect on textiles, they are a cause of concern due to the associated side effects, action on non-target zone, toxicity and water pollution [9].

To minimise the risks associated with the application of synthetic antimicrobial agents, there is a great demand for antimicrobial textiles based on non-toxic and ecofriendly bioactive compounds. Natural biocides used in antimicrobial finishing in textiles, herbs, plant extracts and essential oils play an ever growing demand as they are known for bactericidal and fungicidal properties [10,11,12].

The present study uses the neem oil vapour containing bioactive compounds as antimicrobial agent. Azadirachta indica (neem) is a medicinal plant, and extracts of almost all the parts of the neem tree such as leaves, seed, seed kernel and bark are commonly used for therapeutic purposes [13,14]. Neem oil suppresses the growth of several species of pathogenic bacteria such as *S. aureus*, *E. coli*, S. *typhosa* and all strains of *M. tuberculosis* [15].

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It comprises mainly of triglycerides, large amounts of triterpenoid compounds that are responsible for bioactivity [16]. Neem oil also contains tetranortriterpenoids including fatty acids, nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid, and steroids (campesterol, beta-sitosterol and stigmasterol) [17,18,19].

The antimicrobial efficacy of such fabrics is of primary concern, and it can be improved by enhancing the antimicrobial finish uptake capacity of the fabric. This can be achieved by modifying the fabric surface before processing the fabric with antimicrobial finish. Of the three techniques, namely, chemical, physiochemical and biochemical treatments, the latter two are ecofriendly. Plasma surface modification is a physicochemical technique that is the most effective, efficient and feasible approach to induce physical and chemical changes on textile surface [20,21,22].

The main objective of the present study is to improve the adhesion between the cotton fabric and bioactive compounds present in the neem oil vapour by DC air plasma treatment and to assess the enhancement in antimicrobial activity. An attempt has been made to identify the bioactive compounds present in neem oil vapour and also to understand the reaction with plasma treated cotton fabric.

Materials

Fabric

The cotton fabric was purchased from M/S, Ranjana stores, Coimbatore. The fabric used in the present study is a 100% pure, finished, plain weave 22s warp and 18s weft count with an EPI/PPI ratio of 130:140, surface mass of 140 g and thickness of 0·36 mm.

Cotton, being a cellulosic material (Fig. 1), contains a large number of hydroxyl (OH) groups and a few carboxyl (C═O) groups that induce polarity, making the surface hydrophilic [23].

The three hydroxyl groups attached to C_2, C_3 and C_6 are the reactive sites in cellulose unit. All the three sites are susceptible to both esterification and etherification. Among the three sites, C_6 is the most susceptible to oxidation, whereas in the case of C_2 and C_3, oxidation occurs through bond cleavage [24,25]. During the growth and purification process, the primary hydroxyl group in the cellulose chain oxidises to form 1 glucuronic acid residue to nearly 100 glucosidic bonds [26]. Studies by various groups on diffraction of X-ray by cotton cellulose have revealed that it is semicrystalline [27,28]. The cellulose chains are held parallel to each other by mutual H bond between hydroxyl groups in the crystalline region with the monoclinic crystal structure ($P2_1$: unique axis b) as shown in Fig. 2.

In the amorphous regions, the chains are not parallel due to the absence of hydrogen bond (Fig. 1), and hence, a large number of cellulosic hydroxyl groups are available for reaction [28]. Through chemical, physicochemical and biochemical treatments, highly ordered crystalline region can be transformed into hydrogen bond free amorphous region, thus increasing the number of reactive hydroxyl sites [28].

Neem oil

Neem oil (100% pure) was purchased from Beros Enterprises, Coimbatore. The oil has been prepared using traditional Indian cold press technique. This was used to impart antimicrobial property to the fabric. When neem oil is heated, the respective compounds are liberated at their characteristic vapourisation temperature.

Methods

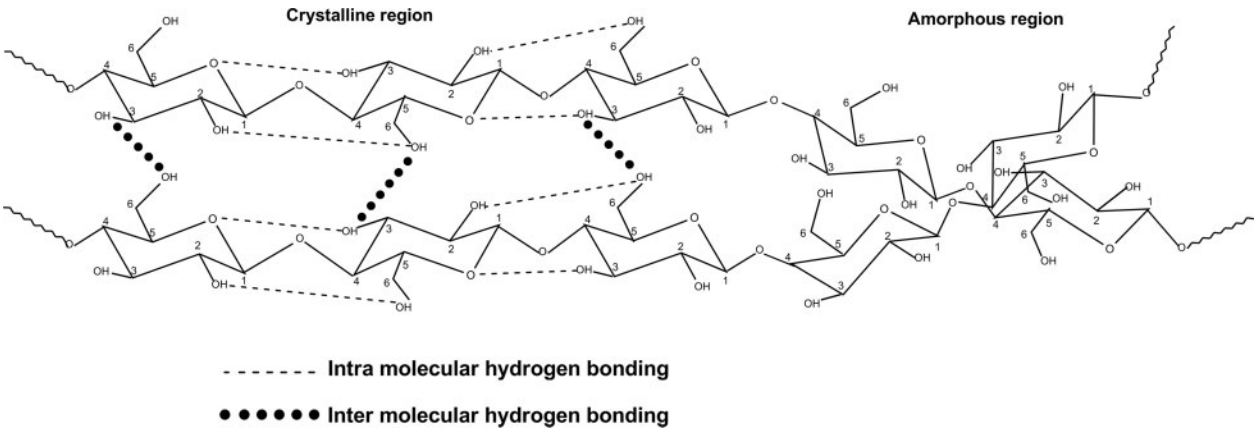
Preparation techniques

Antimicrobial finish treatment

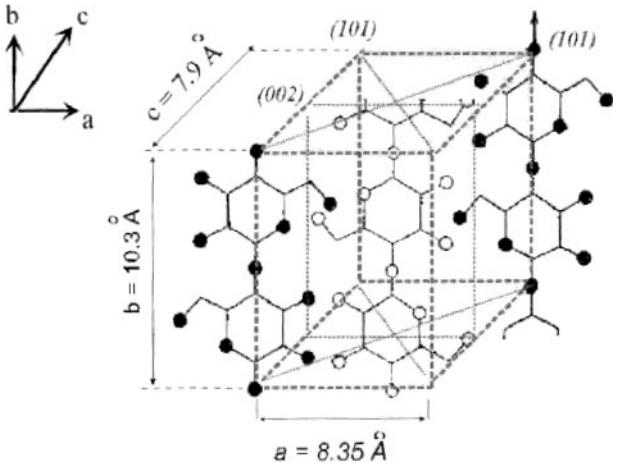
The plasma pretreated fabric was processed with vapours emanating from neem oil (80 mL) in a hot air oven (T26/AO-Technico electric aging oven). The vapour transport distance (distance between oil level and fabric surface) was maintained as 2 cm, and the other process parameters such as the temperature of neem oil (130, 140, 150 and 160°C) and the time of exposure of the fabric to vapour (20, 40, 60, 80 and 100 min) were varied and optimised to obtain maximum antimicrobial efficacy.

Assessment of antimicrobial activity

The sample was subjected to agar diffusion test (SN 195920) [29] to assess the antimicrobial activity. Sterile AATCC bacteriostasis agar was dispensed in sterile petri dish, and 24 h broth cultures of *S. aureus* and *E. coli* were used as inoculums. The test organisms were coated over the surface of the agar plate using a sterile cotton swab. A sample of 2 cm diameter was gently pressed in the centre of the mat culture. The plate was incubated at 37°C for 18–24 h. After incubation, a clear space along the sides of the test material was measured as the zone of inhibition.



1 Structure of cotton cellulose



2 Diagram of unit cell of Cellulose I as derived by Meyer and Misch [40].

The area of inhibition zone is a measure of antimicrobial effectiveness. The zone of inhibition is determined using equation (1):

$$W_C = (T_{tc} - D_t)/2 \quad (1)$$

where W_c is the width of the clear zone of inhibition (in mm), T_{tc} is the width of test specimen and clear zone (in mm), and D_t is the width of test specimen (in mm).

Assessment of antifungal activity

The agar diffusion (AATCC 30) is a qualitative analysis used to assess the antifungal activity of treated fabrics. *Penicilium sp.* and *Trichoderma sp.* were the species used for the study of antifungal activity.

A pinch of fungal culture was inoculated into 50 mL of 3% glucose in Erlenmeyer flasks with few glass beads. The test organism was evenly swabbed on sterile Sabouraud dextrose agar medium (SDA) plates with the culture taken from the mixture. The discs were then distributed evenly with $0{\cdot}2 \pm 0{\cdot}01$ mL of the inoculums using sterile pipette. The inoculated discs were then placed over SDA plate previously swabbed with the culture. The plates were incubated at room temperature for 48–72 h. The zone of inhibition was calculated using equation (1).

DC air plasma treatment

To facilitate better adhesion with a bioactive compound present in neem oil vapour, the fabric sample of dimension 20 cm × 20 cm was pretreated with DC air plasma. A 12 inch DC plasma chamber was used for plasma treatment. The plasma chamber and electrodes were made of stainless steel with an electrode area of 400 cm^2. The gas in the plasma chamber was evacuated using a rotary pump to attain a base pressure of 0·02 mbar, and commercial grade air (RH = 58%) was allowed inside the chamber to maintain a pressure of 0·5 mbar. The pressure was monitored using pirani gauge (Hind Hivac), which is connected to the chamber. The gap between the electrodes was fixed as 4 cm, and the fabric was allowed to float at a distance of 2 cm between the cathode and the anode. The fabric was exposed to plasma for 15 min, which was produced using DC current of 25 mA and a potential of 200 V, i.e. a power density of 0·0125 W cm^{-2} across the electrodes.

Characterisation techniques

Gas chromatography–mass spectrometry studies

The compounds present in the neem oil were identified using gas chromatography–mass spectrometry (GC-MS) analysis. The chemical analysis was carried out using Thermo-GC trace ultra version 5·0, Thermo MS DSQ II. The column used was DB-35-MS capillary standard non-polar column with He as the carrier gas. The temperature was varied from 50°C to 250°C at a rate of 6°C min^{-1}.

Attenuated total reflection–Fourier transform infrared spectroscopy studies

The chemical changes on the fabric surface due to various treatments were analysed using attenuated total reflection–Fourier transform infrared spectroscopy (ATR-FTIR) spectra. The spectra were recorded using a Perkin Elmer (Spectrum100) FTIR spectrometer in the range of 4000–500 cm^{-1} with a resolution of 1 cm^{-1}.

X-ray diffraction studies

An X-ray diffractometer (model: D8 Advance, Bruker, Germany) was used to analyse the crystalline nature and to determine the degree of crystallinity of untreated and plasma treated fabric. Cu K_α radiation of wavelength of 1·54 Å and 40 kV was used for measurement with 2θ values between 10° and 90°.

Segal developed an empirical method for estimating the degree of crystallinity of native cellulose (Cellulose I) [30,31]. According to this method, the amount of crystalline cellulose in the total cellulose content is expressed by the 'crystallinity index' (CI) defined by

$$\mathrm{CI} = 100\left[\frac{\mathrm{I_{002}} - \mathrm{I_{am}}}{\mathrm{I_{002}}}\right] \quad (2)$$

where I_{002} is the intensity of the cellulose principle peak at $2\theta = 22{\cdot}7°$ and I_{am} is the intensity attributed to amorphous cellulose at $2\theta = 18°$.

Scanning electron microscopy studies

Some of the physical changes that the plasma treatment induces on the fabric surface are etching, formation of microcavities and vacancies. The surface morphology of the untreated, plasma treated and neem oil vapour treated fabric samples was analysed using scanning electron microscope (JEOL-EO JSM 6390). Initially, in order to avoid charging effects, the fabric samples were coated with few nanometre thickness of gold. The samples were placed in a scanning electron microscopy (SEM) chamber on a steel stub using conducting tape. The micrographs were recorded for the acceleration voltage of 20 kV with a 2500× magnification at a working distance and spot size of 10 mm and 21 nm respectively.

Assessment of mean pore radius

Dynamic wicking test is used to determine the effective pore radius. The technique relies on describing liquid penetration kinetics inside a material by the Lucas–Washburn equation (equation (3)). The Lucas Washburn equation is derived by combining the poiseuille's method for viscous flow and the Young–Laplace equation for capillarity [32]. Dynamic wicking test (BS 4554) was used to find the wicking height of the

untreated and DC air plasma treated fabric. In this test, a strip of the fabric was suspended vertically with its lower edge in contact with the distilled water. The rise in height of water was measured for different periods of time. The graph is plotted between square of wicking height (L^2) and time taken (t). Using Lucas–Washburn equation, the average pore size of the fabric both before and after plasma treatment was calculated. The slope of the linear plot between L^2 and t provides the mean pore radius of the fabric.

$$L^2 = \left(\frac{R\gamma}{2\eta}\right)t \tag{3}$$

where L is the wicking height, R is the mean pore radius, η is the coefficient of viscosity of the liquid, γ is the surface tension of the liquid and t is the time taken for wicking.

Results and discussion

Antimicrobial activity

The control fabric was treated with neem oil vapour for a fixed time and temperature of 1 h and 150°C, and assessment of antibacterial activity by agar diffusion method against Gram positive organism (*S. aureus*) and Gram negative organism (*E. coli*) is tabulated in Table 1.

Table 1 reveals the suppression of microbial growth just beneath the fabric against *S. aureus*. To improve this antimicrobial efficacy, the fabric surface must be activated to enhance the uptake capacity of neem oil vapour. Surface modification was achieved by preprocessing the fabric with DC air plasma as mentioned in experimental procedure. During neem oil vapour treatment, the operating parameters such as temperature and time of treatment are optimised for maximum antimicrobial efficacy.

Time of treatment is fixed as 1 h, and plasma preprocessed fabrics were treated with vapours of neem oil for different temperatures. The antibacterial activity of processed samples for different temperatures at constant time against *S. aureus and E. coli* is presented in Table 2.

Table 1 Antibacterial activity of untreated and neem oil vapour treated cotton fabric against *S. aureus and E. coli*

Untreated (control) cotton fabric			
		Zone of inhibition/mm	
Temperature (±2°C)	Time/min	*S. aureus*	*E. coli*
150	60	Suppressed growth	Nil

From Table 1, it can be observed that the maximum antimicrobial activity has been achieved for the plasma pretreated fabric exposed to vapour of neem oil, maintained at a constant temperature of 150°C for 60 min. In order to optimise the treatment time, the fabric was processed with neem oil vapour by varying the time between 20 and 80 min by fixing the temperature as 150°C, and antibacterial activity is tabulated in Table 3.

From Tables 2 and 3, it can be confirmed that the fabric exposed to neem oil vapours at 150°C for 60 min exhibits maximum antibacterial efficacy. The values are comparable with the antimicrobial activity of silver nanoparticle treated cotton fabric [33]. This sample was assessed for antifungal activity against *Penicilium sp.* and *Trichoderma sp.*, and the results are tabulated in Table 4.

GC-MS analysis

Identification of the main bioactive compound present in neem oil that contributes to the antimicrobial activity was carried out by comparing the GC retention times and MS data against those of the reference standards [34]. The GC-MS spectrum of neem oil is shown in Fig. 3.

It is evident from Table 5 that fatty acids evaporate at different temperatures. These fatty acids possess a notable activity against various bacteria and viruses [35–37]. Tetradecanoic acid (myristic acid) vapourises at 150 ± 2°C. Data in Table 1 confirm that tetradecanoic acid exhibits a higher antimicrobial activity when compared to other acids, within the temperature range in which the experiment was carried out.

Table 3 reveals that antimicrobial activity (zone of inhibition against *S. aureus and E. coli*) increases with time and reaches a maximum value for a treatment time of 60 min and decreases thereafter. This may be attributed to the fact that when neem oil is maintained at a temperature 150 ± 2°C beyond 60 min, the residual compounds present in the oil react and emanate the vapour of the byproduct, which is not as effective as in the case of tetradecanoic acid.

The results of the studies reveal that plasma treatment has considerably modified the cotton cellulose and has also improved its interaction with neem oil vapour, thus resulting in enhanced antimicrobial activity. Studies on this interaction using the appropriate characterisation techniques and analysis of the results have resulted in a better understanding of the reaction mechanisms.

Reaction mechanism between cotton cellulose and tetradecanoic acid

A comparison between ATR-FTIR spectrum of cotton fabric and the untreated (control) fabric treated with

Table 2 Antibacterial activity of plasma and neem oil vapour treated cotton fabric against *S. aureus* and *E. coli*

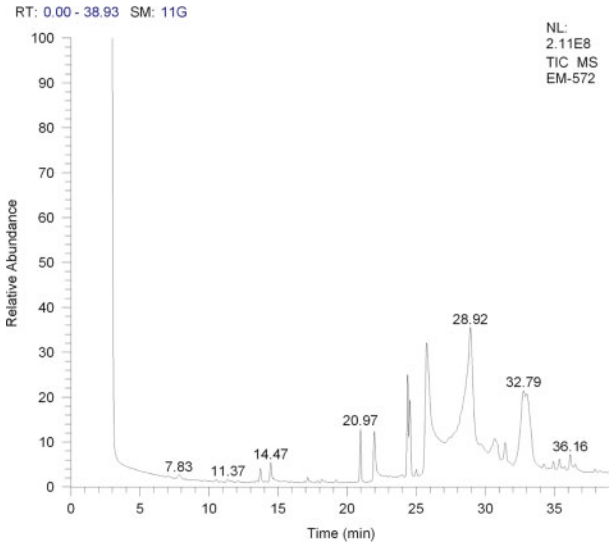
Temperature optimisation			
		Zone of inhibition/mm	
Temperature (±2°C)	Time/min	*S. aureus*	*E. coli*
130	60	Suppressed growth	Nil
140	60	1	Suppressed growth
150	**60**	**6**	**4**
160	60	0·5	Nil

Table 3 Antibacterial activity of plasma and neem oil vapour treated cotton fabric against *S. aureus* and *E. coli*

Time optimisation			
		Zone of inhibition/mm	
Time/min	Temperature/°C	*S. aureus*	*E. coli*
20	150	Nil	Nil
40	150	1·1	Suppressed growth
60	150	6	4
80	150	1·5	1·1

Table 4 Antifungal activity of plasma and neem oil vapour treated cotton fabric against *Penicilium* and *Trichoderma*

Plasma and neem oil vapour treated cotton fabric			
		Zone of inhibition/mm	
Temperature (±2°C)	Time/min	*Penicilium*	*Trichoderma*
150	60	3·5	2·5



3 Gas chromatogram of neem oil

neem oil vapour maintained at 150±2°C for 60 min (Fig. 4) reveals the following.

There is an increase in the intensity of the peaks in the IR spectrum of neem oil vapour treated fabric (Fig. 2*a–c*, inset) pertaining to 2922 cm^{-1} (CH_2 stretch), 2856 cm^{-1} (CH_2 stretch), 1743 cm^{-1} (C═O str) and 1235 cm^{-1} (C—O str). This can be attributed to the esterification that occurs between the hydroxyl and carboxyl groups of cellulose and tetradecanoic acid. The peak corresponding to C═O group in esters normally appears at a slightly higher frequency when compared to aldehydes and ketones. In this case, a shift towards the lower frequency can be attributed to the electron releasing effect of the acyl chain (+ I effect), which tends to weaken the C═O bond strength, shifting the peak towards a lower frequency.

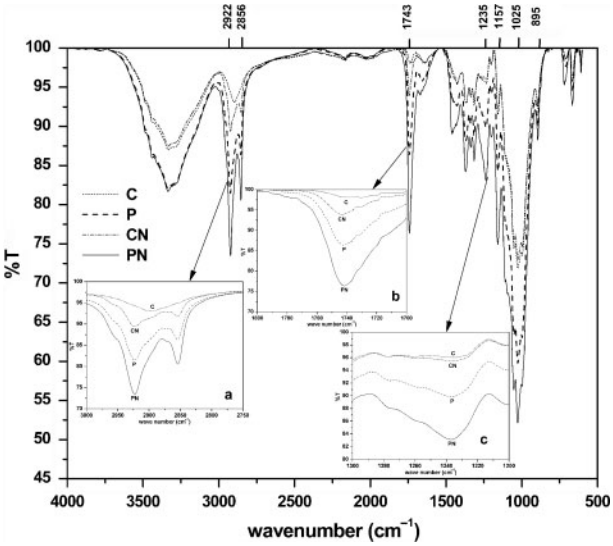
The ATR-FTIR analysis thus seems to confirm the formation of an ester bond between plasma treated cotton cellulose and tetradecanoic acid. This is a heterogeneous esterification, where tetradecanoic acid is in a gaseous state and the fabric containing OH groups is in a solid state. Hence, the reactivity of acid molecules would be higher and the water molecules formed escape as water vapour at 150°C, which favours the bonding of ester with cellulose. Further, the remaining parts of the tetradecanoic acid contain no active sites. Rather, it contains only acyl chain. However, the acyl chain may assign a configuration that could fit on the active surface of microorganisms, which breaks the weaker ester bond with cellulose, leaches from the fabric and kills the microorganism.

Effect of DC air plasma on structure of cotton cellulose

In the present study, in order to make the surface more reactive before neem oil vapour treatment, the fabric was treated with air plasma. The ATR-FTIR spectra of untreated (control) and plasma treated fabric (Fig. 4) were compared to investigate the role of plasma surface modification in enhancing the uptake of tetradecanoic acid.

Table 5 Compounds eluted from neem oil at different temperatures

Retention time	Temperature/°C	Compound	Molecular formula
7·83	97	3-Methyl benzo furan	C_9H_8O
11·37	119	7,9-Dodecadien-1-ol	$C_{12}H_{22}O$
13·72	132	Dodecanoic acid, methyl ester (lauric acid)	$C_{13}H_{26}O_2$
14·47	137	Dodecanoic acid (lauric acid)	$C_{12}H_{24}O_2$
17·16	152	Methyl tetradeconoate (myristic acid)	$C_{15}H_{30}O_2$
17·87	157	4-t Butyl 3-cyano 6-methyl- 2(1H) pyridinone	$C_{11}H_{14}N_2O$
18·16	160	Tridecanoic acid	$C_{13}H_{26}O_2$
20·97	175	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
21·97	181	Hexadecanoic acid	$C_{16}H_{32}O_2$



4 ATR-FTIR spectra of control (C), DC plasma treated fabric (P), control + neem oil vapour treated fabric (CN) and plasma + neem oil vapour treated fabric (PN)

The comparison resulted in the following significant observations:

1. Peaks pertaining to the respective functional groups that are present in cellulose do seem to exist in the spectrum of plasma treated cotton cellulose, and no additional peaks pertaining to new functional group are found in the spectrum of plasma treated fabric
2. There is an increase in the intensity of peak at 1743 cm^{-1} corresponding to C═O stretching and 1025 cm^{-1} corresponding to C—O stretching in the spectrum of plasma treated cotton cellulose.
3. No change was observed in the intensity of peaks corresponding to 1157 and 895 cm^{-1}, which represent asymmetric bridge C—O—C and C$_1$—O—C$_4$ (β glucosidic bond) of cellulose chain.

An analysis of the above mentioned observations reveal that air plasma treatment has resulted in the oxidation of cellulose, which in turn has led to an increase in the concentration of C═O groups. C_1, C_2, C_3 and C_6 are four possible sites for oxidation of cellulose [38,39]. The observations discussed also seem to confirm that C_6 is the site where oxidation occurs due to DC air plasma treatment and not the other three. This is because oxidation at those sites would occur through ring opening and chain scission. The peaks corresponding to 1157 and 895 cm^{-1} in both the spectra are similar in intensity, confirming the absence of ring opening or chain scission.

To investigate the effect of oxidation on the degree of crystallinity of cellulose, X-ray diffraction (XRD) analysis was carried out on untreated and plasma treated cotton cellulose. Figure 5 shows the X-ray diffractogram of the untreated and plasma treated cotton fabric.

The crystal structure of cellulose is monoclinic with three principal planes of reflection, namely, (002), (101) and (10$\bar{1}$). It is clear from the unit cell given in the inset of Fig. 4 [28,40] that the interchain hydrogen bonding lies in the (002) plane. Both samples show similar XRD patterns, which imply that the crystal structure of cellulose is not subject to any change. However, to understand if the plasma treatment had any effect on crystallinity, degree of crystallinity has been calculated for both the samples using equation (2).

The CIs for control and plasma treated cotton fabric are 84·7% and 81·1% respectively. There is a noticeable difference between the CI value of the control and plasma treated cotton fabric. The results reveal a decrease in the degree of crystallinity when cotton cellulose is treated with DC air plasma, which is a consequence of the action of plasma active particles. Further, the IR spectrum reveals the oxidation of cellulose.

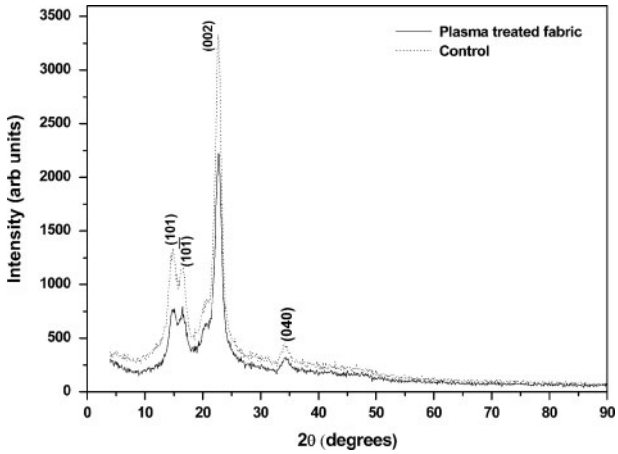
From the analysis, it can be inferred that oxidation reaction breaks the interchain hydrogen bonding, i.e. parallel alignment of cellulose chain. This results in an increase in amorphous (non-crystalline) regions, as shown in Fig. 6. The number of OH reactive group increases with a decrease in crystallinity of the sample [28]. This results in a subsequent number of reactions with tetradecanoic acid in the amorphous region, where accessible hydroxyl groups are present. These reactions in turn increase the antimicrobial activity of the plasma treated samples when compared to the control sample.

Reaction between plasma treated cotton cellulose and tetradecanoic acid

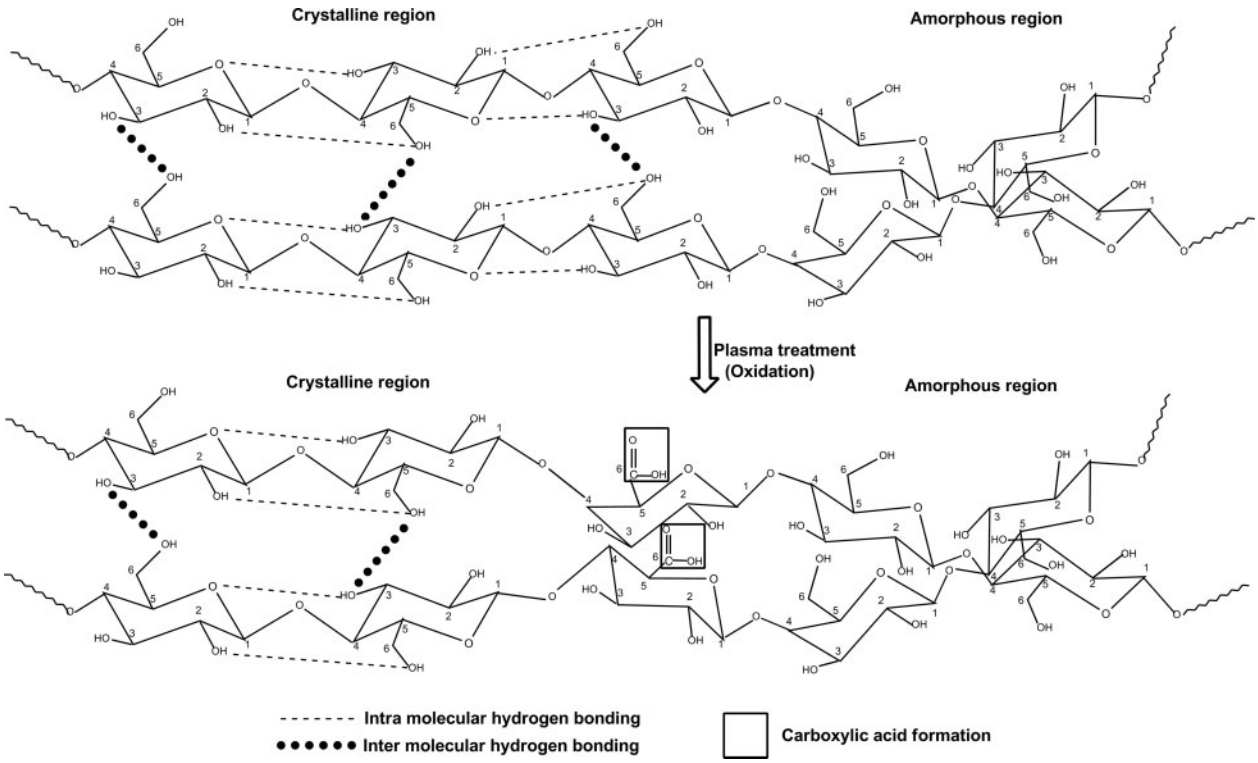
The reaction that takes place between plasma treated cotton cellulose and tetradecanoic acid was analysed by comparing the ATR-FTIR spectra of DC air plasma treated cotton cellulose and the one treated with plasma and neem oil vapour (Fig. 2). The IR spectrum of plasma and neem oil vapour treated cotton cellulose shows four peaks at 2922 cm^{-1} (CH_2 stretch), 2854 cm^{-1} (CH_2 stretch), 1743 cm^{-1} (C═O str) and 1235 cm^{-1} (C—O str) that are relatively intense when compared to those peaks present in the spectrum of plasma treated cotton cellulose (Fig. 2*a–c*, inset). This can be attributed to the formation of ester resulting from the reaction among all possible reactive hydroxyl sites of plasma treated cotton cellulose and tetradecanoic acid. The reaction mechanism is shown in Fig. 7.

Effect of DC air plasma on morphology of cotton cellulose—SEM analysis

SEM micrographs of untreated, DC air plasma treated and neem oil vapour treated samples were observed with a magnification of 1500 × and are presented in Fig. 8.



5 X-ray diffractogram of control and plasma treated sample

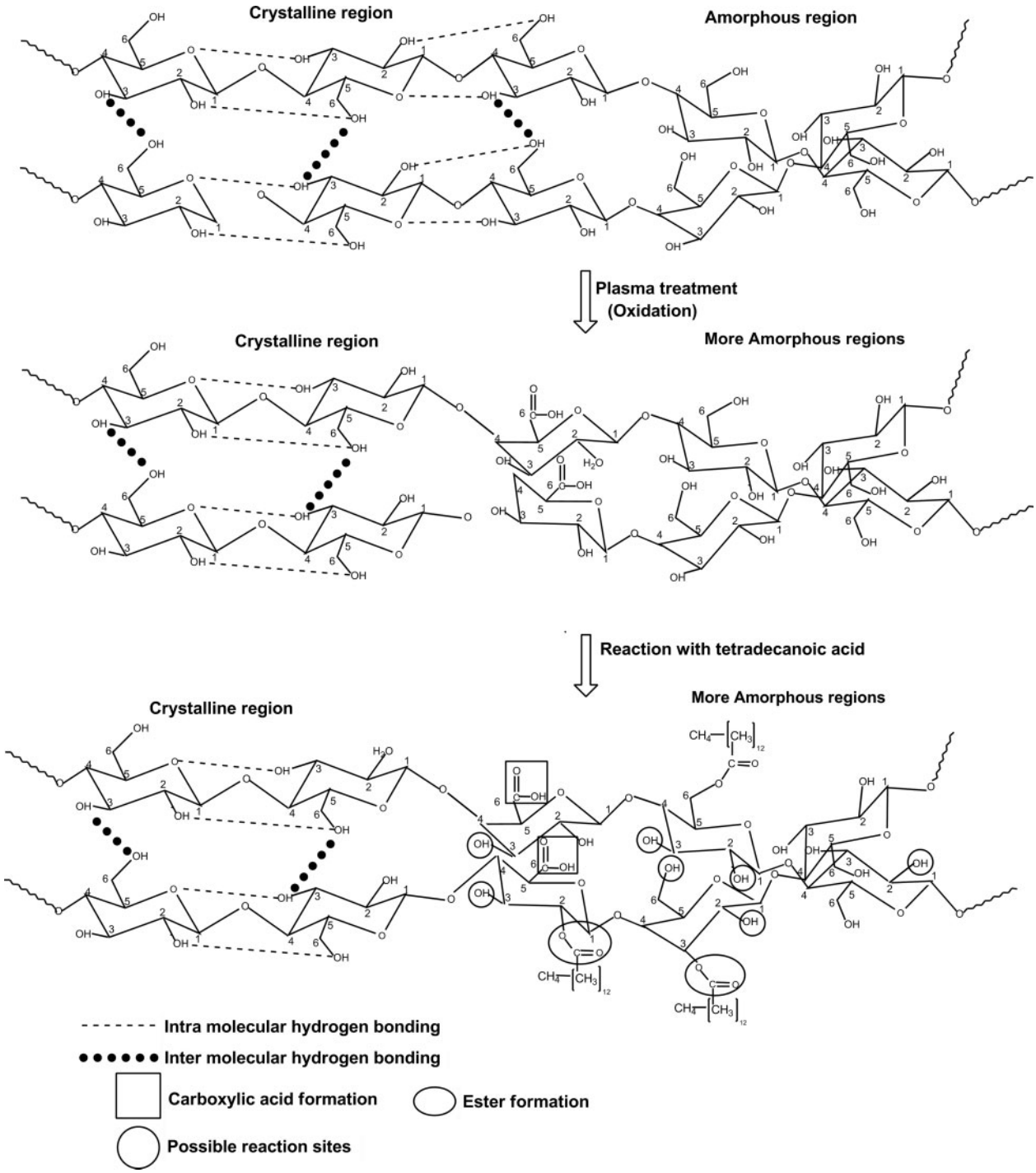


6 Representation for decrease in CI due to DC air plasma treatment

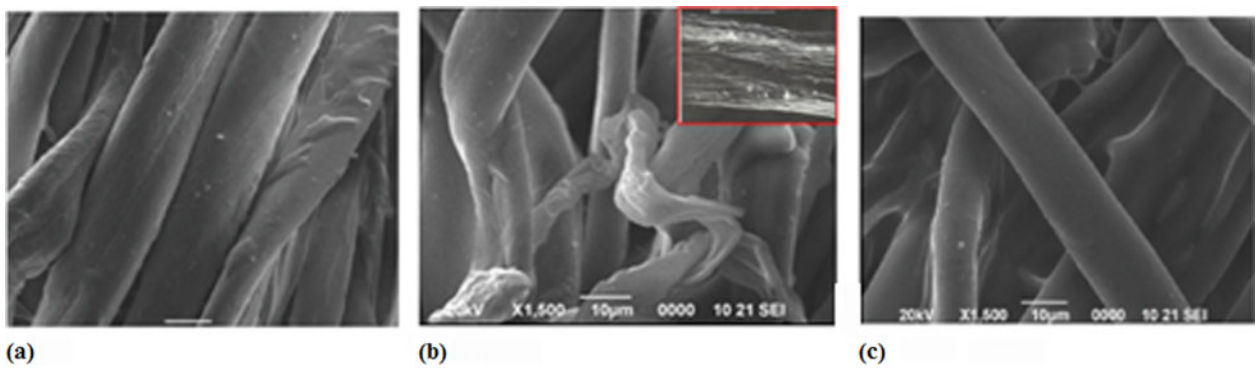
It can be observed that, in addition to the chemical reactions that occur on fabric due to plasma treatment, the surface is also subject to physical changes, namely etching, creation of microcavities and an increase in existing pore dimension. These changes can be explained by comparing the surface morphology and pore size of the untreated and plasma treated fabric samples. The micrographs of untreated and plasma treated fabric (Fig. 8*a* and *b*) reveal that the plasma treatment has resulted in etching the surface of the fabric, thus increasing the surface roughness [41]. The mean pore radii of control and plasma treated samples are calculated using Lucas–Washburn equation, and the values are 0·1292 and 0·3364 μm. The results of mean pore radius reveal that the bombardment of plasma particles on the fabric surface increases the existing mean pore size, thus improving the capillarity of cotton fabric. These results are in accordance with those obtained by Naveneetha Pandiyaraj and Selvarajan [42].

The vapour in contact with the plasma treated fabric condenses on the surface and makes it smooth (Fig. 8*c*). The presence of the microcavities is responsible for an increase in spreading of oil vapour on the hydrophilic surface due to capillarity, thus promoting the reaction with the hydroxyl sites, which is confirmed by an increase in antimicrobial efficacy of DC air plasma pretreated cotton fabric when compared to the untreated fabric.

The above studies validate that the interaction between plasma particles and cotton fabric simultaneously improves the fabric's capillary action and also increases the concentration of reactive hydroxyl site through etching and oxidation respectively. This synergetic effect promotes fabric's neem oil vapour uptake



7 Reaction mechanisms between DC air plasma treated cotton cellulose and neem oil vapour



8 SEM images of *a* untreated cotton fabric, *b* DC air plasma treated cotton fabric and *c* neem oil vapour treated cotton fabric

capacity and its reaction with tetradecanoic acid present in the neem oil vapour, thus enhancing the antimicrobial activity of the fabric.

Conclusion

The fabric treated with DC air plasma and subsequent exposure to neem oil vapour maintained at 150 ± 2°C for 60 min was found to exhibit an enhanced antimicrobial efficacy when compared to that treated with neem oil vapour alone. A GC-MS analysis of neem oil reveals the presence of tetradecanoic acid in the vapour released at 150 ± 2°C, which is responsible for the antimicrobial activity. Esterification of cotton cellulose, when exposed to neem oil vapour, has been confirmed through ATR-FTIR analysis. The DC air plasma pretreatment given to fabric to improve its interaction with the neem oil vapour has lead to the oxidation of cotton cellulose, which in turn has resulted in a decrease in degree of crystallinity. These observations have been confirmed by ATR-FTIR and XRD analysis. The decrease in crystallinity seems to have paved the way for the more reactive hydroxyl sites to interact with neem oil vapour. In addition, the plasma treatment has resulted in increasing the surface roughness and dimensions of microcavities present in the fabric. These observations have been confirmed through SEM analysis and also by calculating the average pore size both before and after plasma treatment using Lucas–Washburn equation. This has led to improved capillary action. The synergetic effect of improved capillary action and an increase in concentration of reactive hydroxyl sites has enhanced the neem oil vapour uptake by the fabric and hence the antimicrobial activity. When the plasma treated fabric is treated with neem oil vapour maintained at 150 ± 2°C, the tetradecanoic acid present in the vapour reacts with the hydroxyl group of the plasma treated cotton cellulose to form ester, which has been confirmed through ATR-FTIR analysis. The surface morphology of plasma and vapour treated fabric reveals the smoothening of the plasma treated fabric surface due to the adherence of vapour on the fabric.

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