A Study of Antimicrobial Property of Textile Fabric Treated with Modified Dendrimers

Subhas Ghosh,[1] Sangeeta Yadav,[1] Nadarajah Vasanthan,[2] Gabriela Sekosan[2]

[1]*School of Technology Studies, Eastern Michigan University, Ypsilanti, Michigan 48197*
[2]*Chemistry Department, Long Island University, New York, New York 11201*

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ABSTRACT: Dendrimers have been used as a vehicle to develop the antimicrobial properties of textile fabrics. We have taken advantage of the large number of functional groups present in the regular and highly branched three-dimensional architecture of dendrimers. In this study, the poly(amidoamine) (PAMAM) G-3 dendrimer was modified to provide antimicrobial properties. Following a procedure similar to what is suggested in the literature, PAMAM (G3) with primary amine end groups was converted into ammonium functionalities. The modification was then confirmed by FTIR and ^{13}C-NMR analysis. Dendrimers have unique properties owing to their globular shape and tunable cavities, this allows them to form complexes with a variety of ions and compounds; and also act as a template to fabricate metal nanoparticles. $AgNO_3$–PAMAM (G3) complex as well as a MesoSilver–PAMAM (G3) complex were formed and these modified dendrimers were characterized by a UV–Visible spectrophotometer to study the complex formation. Modified dendrimers were applied to the Cotton/Nylon blend fabric. SEM and EDX analysis were performed to study the dispersion of silver nanoparticles onto the fabric. An antimicrobial test of the treated-fabric against *Staphylococcus aureus* exhibited significant biocidal activities for each type of modified-dendrimer. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 716–722, 2010

Key words: dendrimers; silver nanoparticles; biocides; antimicrobial

INTRODUCTION

Dendrimers are a class of low-molecular weight highly branched polymers with several functional groups that have a central core and terminal end groups. Tomalia et al.[1] discovered dendrimers in the early 1980's and they are generally prepared using either a divergent method or a convergent method. In the divergent method, dendrimers grow outward from multifunctional core molecules, whereas, in the convergent method, the dendrimer is constructed stepwise starting from the end group and moving inward.[2] Unlike the synthesis of linear polymers, the size and molecular mass of dendrimers can be specifically controlled during synthesis. The dendrimers exhibit an exponential increase in functional groups with generations and they are usually monodispersed macromolecules with a regular and highly branched three-dimensional architecture.[1–6] It has been shown that highly branched molecules behave differently from conventional polymers.[1–10] Each layer in a dendrimer makes up a single generation, the structure of one such a dendrimer, poly(amidoamine) (PAMAM) is shown in Figure 1.[5]

The modification of dendrimers has been of great interest to scientists in various applications. Surface modification of dendrimers has been carried out for such biological applications as gene therapy, catalysts, and drug delivery systems.[11–19] The nature of the outer functional groups determines the solubility and reactivity of the dendrimers. Acetylation and quaternization of dendrimers were reported recently.[20,21] Monovalent and bivalent biocides are widely used for biomedical applications, however, polyvalent biocides are lacking. It is expected that polyvalent biocides would have high activities compared with mono or bivalent biocides.[22-25] A number of polymeric biocides were prepared and tested for their antimicrobial properties. Dendrimers have been used to prepare polyvalent biocides. It was believed that dendrimers with amine functional groups could be converted to effective antimicrobial agents due to their dense primary amine functional groups. It is well known that quaternary ammonium salts are an effective antimicrobial agent.[26,27] Dendrimers can also be tailored to generate tunable cavities with ammonium or phosphonium functionalities.

Dendrimers have some unique properties because of their globular shape and tunable cavities. It has also been shown that dendrimers are able to form complexes with a variety of ions and compounds and act as a template to fabricate metal nanoparticles. Synthesis of silver/dendrimer nanocomposite and silver complexes was reported.[28–30]

Correspondence to: S. Ghosh (sghosh@emich.edu).
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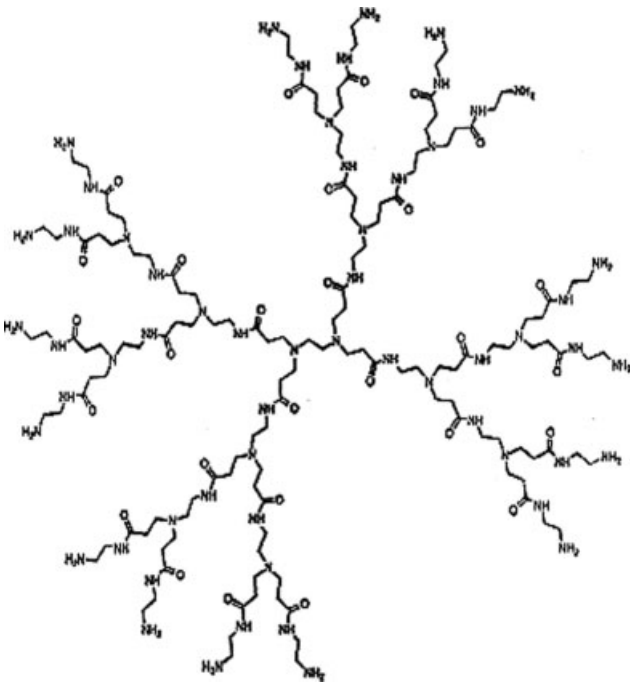


Figure 1 Structure of poly(amidoamine) (PAMAM).[5]

It has been shown that toxicity is very low at a low concentration of dendrimers nanocomposite; however, higher toxicity was observed at a relatively high concentration.[29] A dendrimer template is able to control the size of the nanoparticles. The structure of these materials is different from those silver colloids with dendrimers as a stabilizer. A procedure was reported in the literature about the preparation of an internal silver–dendrimer nanocomposite.[28]

Antimicrobial agents are mainly made up of small molecules. The use of antimicrobial polymers was explored to some extent,[24,31] and it has been shown that an antimicrobial polymer has a high efficiency compared with existing antimicrobial agents. The use of dendrimers as an antimicrobial agent could be beneficial because of their unique structures. This article describes a study of modified-dendrimers with ammonium functionalities and producing a nanoparticles–poly(amidoamine) complex. These materials were applied to the textile fabric to study antimicrobial activities.

EXPERIMENTAL

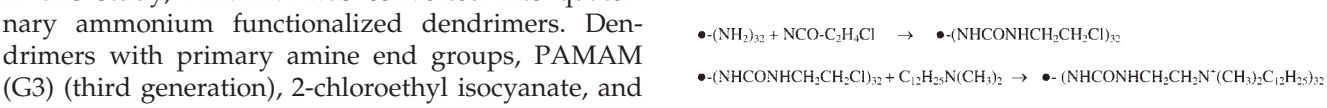
Synthesis of dendrimers into quaternary ammonium functionalities

In this study, PAMAM was converted into quaternary ammonium functionalized dendrimers. Dendrimers with primary amine end groups, PAMAM (G3) (third generation), 2-chloroethyl isocyanate, and dimethyl dodecylamine were purchased from the Sigma Aldrich Chemical Company. All HPLC grade solvents were also purchased from Sigma Aldrich. All of these chemicals were used as received. The functionalization was carried out in two steps, as suggested in the Ref. 21. The first step was introducing chlorine functionalities to the dendrimer by reacting primary amine groups of the dendrimers with 2-chloroethyl isocyanate. PAMAM (G3) (0.500 g) dendrimers were obtained by drying 2.5 g of 20% PAMAM (G3) in a methanol solution. PAMAM (1 equivalent) was dissolved in 2-methyl-2-pyrrolidinone anhydrous in a dried round bottom flask. 2-chloroethyl isocyanate (1.1 equivalent) was added, drop by drop, in ambient temperature and stirred at room temperature for 12 h to complete the reaction.

These chlorine-substituted dendrimers can react with tertiary amines to form quaternary ammonium salts. After the mixture was stirred, 1 g (5 equiv.) of dimethyl dodecylamine was added to the chlorine-substituted dendrimer (PAMAM). The solution was slowly heated to 80°C and refluxed for 72 h. After the reaction, the mixture was cooled to room temperature. The reaction mixture was concentrated and poured into acetone for precipitation. The mixture was filtered and dried in a vacuum oven for further analysis. The product was a yellow solid material and the yield was $\sim$ 40%. The preparation of quaternary salt is shown in Scheme 1.

Silver particles–dendrimers complex preparation

Silver nitrate was purchased from the Sigma Aldrich Chemical Company and used as received, without further purification. The concentration of the dendrimer ($\sim 0.03M$) and the concentration of the silver ions ($1.065 \times 10^{-3}M$) were kept constant. Silver nitrate was selected for the silver–dendrimer complex preparation because of its good solubility both in water and methanol. To ensure identical silver concentrations, 0.1809 g of $AgNO_3$ was dissolved in 10 mL of deionized water (DI water). This solution was added to 1 mL of PAMAM (G3) dendrimer solution (20% w/w in methanol) drop by drop while stirring, at an ambient temperature. The metal ion/dendrimer ratio was determined by the ratio of metal ion moles per dendrimer molecule. A similar complex preparation was carried out by Ottaviani et al.[28] shows that the complexion of the metal ion does not depend on the concentration but depends mainly on the dendrimer/metal ion ratio and generation.



Scheme 1 Synthesis of quaternary ammonium salt of 3rd generation PAMAM.[21]

MesoSilver–dendrimer nanocomposite preparation

MesoSilver particles were obtained from Purest Colloids. MesoSilver (Silver colloids) is made up of 0.9999 pure silver subnanometer sized particles suspended in pure deionized water; referred to as colloidal silver. The silver particles in MesoSilver typically measure 0.65 nm or less in diameter, this is slightly larger than twice the diameter of a silver atom. Each silver nanoparticle consists of ~ 9 silver atoms. These small particles create a rather high concentration of particles, resulting in the high-particle surface area of silver colloid. It has been demonstrated[2] that dendrimer nanocomposite can be prepared from $(Ag^{+})_{32}$–PAMAM dendrimer by photolysis. To obtain the complete conversion of silver ions into Ag^{0}, X-ray radiation was applied at the rate of 1.5 Gy/min. We applied UV treatment to $(Ag^{+})_{32}$–PAMAM dendrimer complex because we were unable to obtain a silver nanocomposite. This may be attributed to a lack of the level of energy needed to form the complex in our UV source. We used another method to obtain a dendrimer–silver nanocomposite; as follows.

The total concentration of N ligands were kept constant ($\sim 0.03M$) but the concentration of MesoSilver was varied to modify the concentration of nanoparticles. Three different nanocomposites were prepared by adding a MesoSilver suspension to 1 mL of PAMAM dendrimer solution (20% w/w in methanol) drop by drop, while stirring. The amount of dendrimers and MesoSilver used for the preparation of the complex is given in Table I along with the concentration of silver.

Application of functionalized-PAMAM and silver–PAMAM complex on textile fabric

These antimicrobial agents were applied onto 50/50, Nylon/Cotton blend woven fabric evenly, using a laboratory knife through out the surface of the fabric, to simulate a surface coating process. The samples were dried for 6 h under ambient condition.

RESULTS AND DISCUSSIONS

Analysis of modified dendrimers with ammonium functionalities

PAMAM dendrimer (G2) has 16 primary amine functionalities. To increase the effectiveness of the antibacterial activities, we used 3rd generation PAMAM (G3) dendrimers that usually consist of 32 primary amine functional groups. Reaction was monitored by FTIR spectroscopy and NMR spectrometry. An IR spectrum of PAMAM (G3) in the region from 2700 to 3500 cm^{-1} is shown in Figure 2. The IR spectra of PAMAM dendrimer shows a broad band at 3300 cm^{-1}, assigned to N—H stretching vibration and bands around 2800 to 3000 cm^{-1} are attributed to C—H stretching. Figure 2(a) illustrates the IR spectrum for modified dendrimers and shows that strong bands exist in the C—H region, suggesting that long alkyl groups are added to the PAMAM dendrimer. Figure 3 shows the IR spectra of PAMAM and of the modified-PAMAM, including the region from 700 to 1800 cm^{-1}. Comparison of the spectra of PAMAM before and after modifications confirms the conversion of PAMAM into salt. For example, the band at 1645 cm^{-1} is assigned to C=O stretching vibrations and the band at 1553 cm^{-1} is attributed to N—H bending in Figure 3(b). These bands show clear shifts in the modified-spectrum of dendrimers, Figure 3(a), as a consequence of functionalization.

TABLE I
Dendrimers to MesoSilver Ratios Used in the Preparation of Nanocomposite

Materials	Volume (mL)	Concentration
PAMAM dendrimer	1	0.03*M*
MesoSilver	1, 2, 3	37, 50, and 56 ppm, respectively

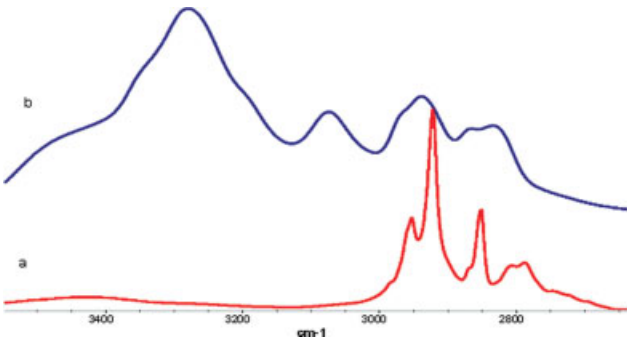


Figure 2 FTIR Spectrum of (a) Modified-PAMAM and (b) Unmodified-PAMAM for ammonium functionalities covering the region 2700^{-1} to 3500^{-1}. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Detailed structural analysis and absolute conformation of the conversion can only be carried out using ^{1}H- or ^{13}C-NMR spectroscopy. It was found that the product was soluble in methanol, and therefore, ^{13}C-NMR spectra were obtained using deuterated methanol as a solvent. The ^{13}C-NMR spectrum of neat PAMAM dendrimers is shown in Figure 4, and of modified dendrimers is shown in Figure 5. Figure 4 shows a resonance at 173 ppm that can be attributed to the carbonyl carbon of the original PAMAM dendrimer. Figure 5 shows two resonances at 173 and 160 ppm, in the region, confirming the functionalization of PAMAM dendrimers. A chemical shift at 160 ppm may be attributed to urea carbonyl (NH—CO—NH) in the quaternary ammonium salt, whereas the resonance at 173 ppm is a carbonyl

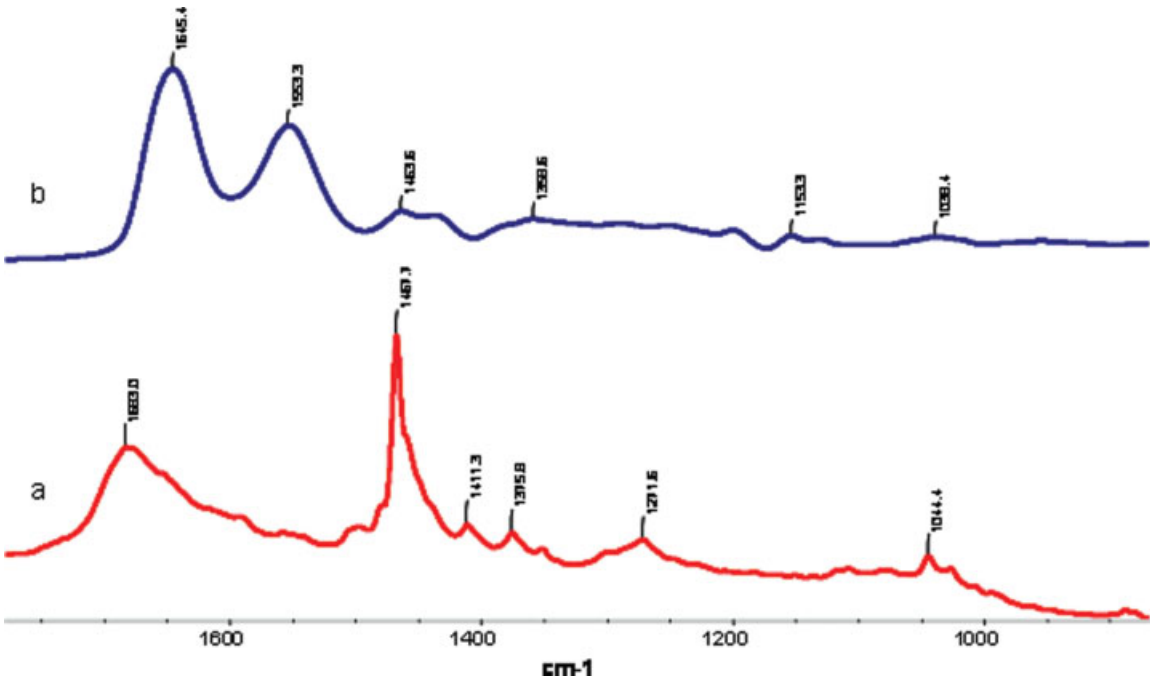


Figure 3 FTIR Spectrum of (a) Modified-PAMAM and (b) Unmodified-PAMAM for ammonium functionalities covering the region 700^{-1} to 1800^{-1}. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

carbon of PAMAM dendrimers. Resonances associated with CH_2 carbons in the neat dendrimer appear in the region between 30 and 50 ppm, Figure 4. However, Figure 5 shows additional peaks for ammonium functionalized dendrimers in the region from 15 to 60 ppm, which is not seen in a ^{13}C-NMR spectrum of neat dendrimer, suggesting that additional CH_3 and CH_2 groups are incorporated in the dendrimer structure.

Analysis of silver ($AgNO_3$) particles–dendrimers complex

$(Ag^+)_{32}$PAMAM, silver nitrate, and pure PAMAM were characterized by a UV–Visible spectrophotometer (Perkin-Elmer Lambda 25), at room temperature between the wavelength range of 200 and 700 nm, using a quartz cell ($L = 1$ mm). Figure 6 shows the UV–Visible spectra of neat $AgNO_3$, a dendrimer, and Silver-PAMAM complex. It is observed in Figure 6 that a new absorption band near 300 nm appears for the Silver–PAMAM complex. This is absent for both pure dendrimers and $AgNO_3$, indicating the formation of the complex. It could, therefore, be possible to use this peak to confirm the Silver–PAMAM complex formation. To check the applications on the fabric treated with Silver–PAMAM, the sample was examined under an SEM that exhibited good dispersion on the fabric surface as seen in Figure 7. The observed particles were mainly identified as silver using energy dispersive X-ray spectrometry (EDX), as shown in Figure 8.

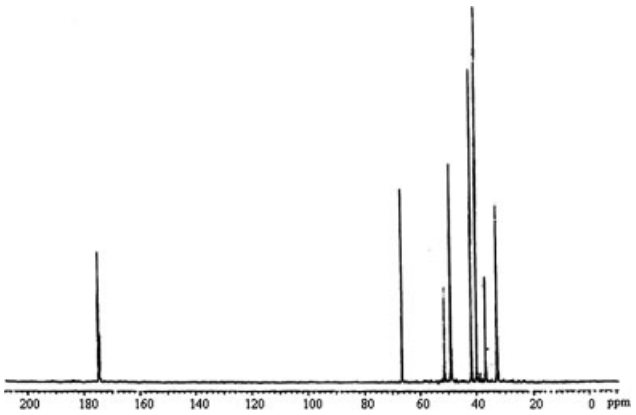


Figure 4 ^{13}C-NMR spectrum of PAMAM.

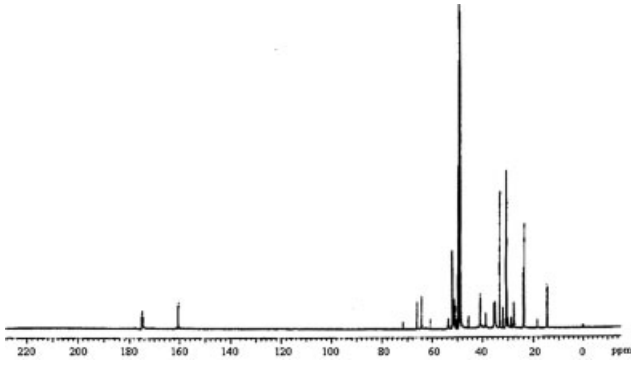


Figure 5 ^{13}C-NMR spectrum of modified-PAMAM.

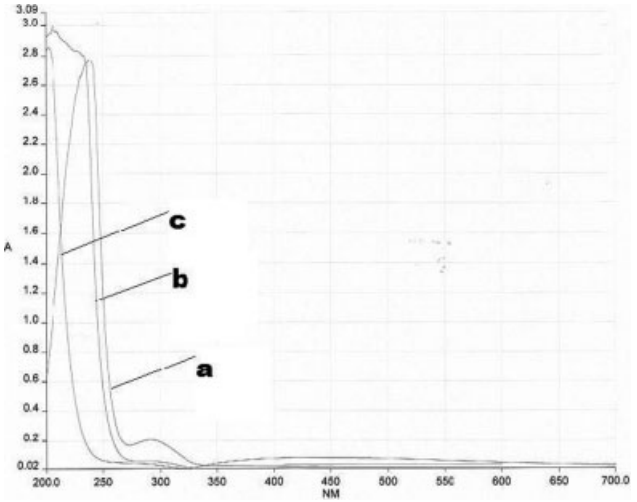


Figure 6 UV–Visible spectra of (a) $(Ag^{+})_{32}$PAMAM, (b) $AgNO_3$, and (c) pure PAMAM.

Analysis of MesoSilver–dendrimer nanocomposite

PAMAM/Ag^0 nanocomposite was previously formed by irradiating $(Ag+)_{32}$ PAMAM complex. These nanocomposites are usually external nanocomposites due to the nature of polar groups in the periphery. This article describes the formation of internal nanocomposites for the first time using MesoSilver. The UV–Visible spectra of PAMAM dendrimer and its nanocomposite were obtained on a Perkin-Elmer Lambda 25 spectrophotometer, at room temperature, between 200 and 700 nm and using a quartz cell ($L = 1$ cm); as shown in Figure 9. It has been shown[28] that a band at 410 nm is a characteristic peak for a silver–dendrimer nanocomposite. Figure 9 shows a peak at 410 nm, suggesting the formation of a nanocomposite. Increase in absorbance at 410 nm was previously attributed to either an increase in concentration or size of the Ag^0 nanoparticles. It is also evident that the peak of absorbance at 410 nm increases slightly with the increasing concentration of Ag^0. The SEM image of MesoSilver–dendrimer composite treated-fabric can be seen in Figure 10. Figure 10 shows the presence of silver nanoparticles, although it appears that the amount of MesoSilver deposits is lower than found in the $AgNO_3$–dendrimer complex. The EDX spectrum of the surface of the fabric containing the MesoSilver–dendrimer complex is illustrated in Figure 11 and further confirms the presence of silver particles on the specimen surface.

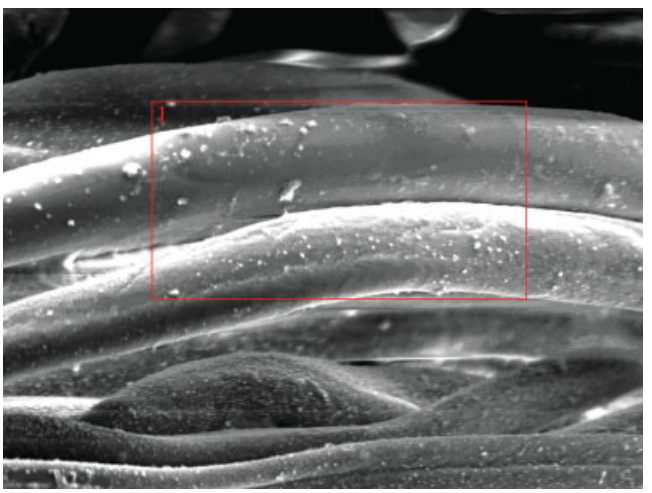


Figure 7 SEM image of the fabric treated with silver/dendrimers complex at 1200 magnification (10.0 kV and 10 mm distance). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

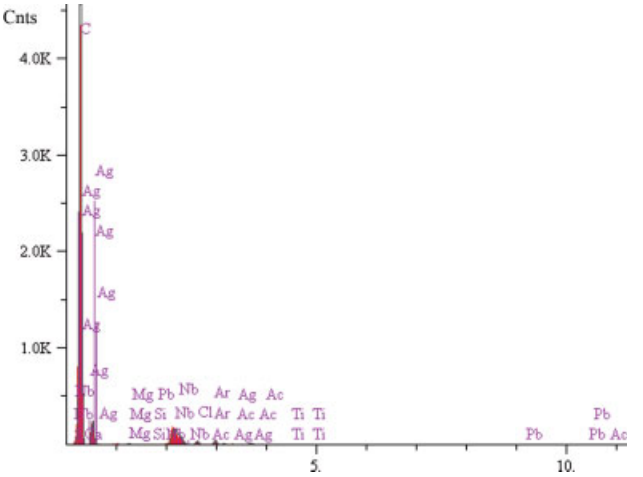


Figure 8 EDX spectra of fabric surface treated with silver($AgNO_3$)/dendrimer complex. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Evaluation of antimicrobial property

The antimicrobial properties of the specimens were tested using an AATCC method-147.[31] The test

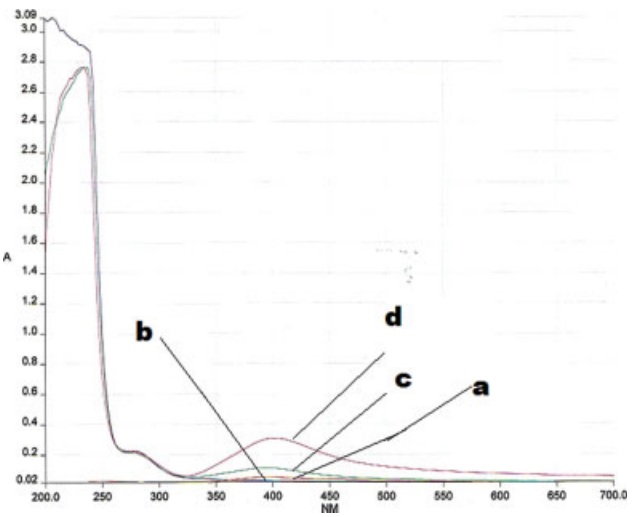


Figure 9 UV–visible spectra of (a) Ag nanoparticle, (b) pure PAMAM dendrimer, (c) Ag^0PAMAM dendrimer (37.5 ppm), and (d) Ag^0PAMAM dendrimer (50 ppm). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

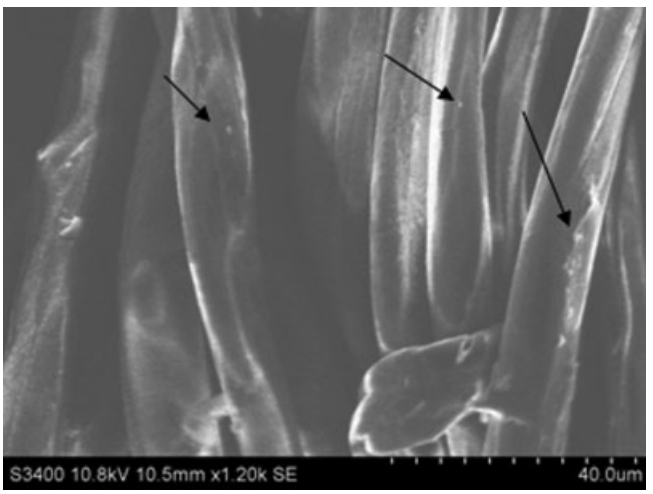


Figure 10 SEM image of MesoSilver–dendrimer composite treated-fabric (1200 mag).

objective was to determine the antibacterial activity of diffusible antimicrobial agents on treated textile fabric. In this method, the agar surface is inoculated by making a parallel streak, and then the sample is pressed onto the plate inoculated. After incubation, an estimate of activity, in that the growth of the inoculum organism decreases from one end of each streak to the other and from one streak to the next. The size of the zone of inhibition and the narrowing of the streaks caused by the presence of the antibacterial agent permit an estimate of the residual antibacterial activity.

One loopful of the diluted inoculum was streaked on an agar plate. The test specimen is gently pressed transversely across the agar surface, which is incubated at 37 ± 2°C for 18–24 h. The incubated plate was examined for the interruption of growth along the streaks of inoculum beneath the specimen and for a clear zone of inhibition beyond its edge. The average width of a zone of inhibition, along a streak, on either side of the test specimen is calculated using the following equation[31]:

$$W = (T - D)/2$$

where:

W = width of clear zone of inhibition in mm.

T = total diameter of test specimen and clear zone in mm.

D = diameter of the test specimen in mm.

There cannot be any bacterial colonies directly under the sample in the contact area to be considered acceptable antibacterial activity. The test bacteria were *Staphylococcus aureus* AATCC 6538, Gram positive organisms. The antimicrobial test data is presented in Table II.

All fabric specimens treated with various antibacterial agents exhibited significant biocidal activities with varying degrees of zones of inhibitions, whereas the untreated-fabric specimen did not show any antimicrobial activities. The AATCC-145 is a qualitative test method. The difference in zones of inhibition does not necessarily mean that a specimen is more or less biocidal. The zone of inhibition depends on the migratory property of the antibacterial agent to diffuse into the agar; hence, it does not depend only on the strength of the biocidal agent. We intend to bind the antibacterial agent very strongly to the fabric to withstand laundering. The differences observed in the zones of inhibition are not necessarily only related to the strength of antimicrobial activity. However, all treated samples did show satisfactory antimicrobial activities. It is now generally accepted that the mode of biocidal action of the ammonium groups follows a sequence of activity: (i) adsorption of ammonium groups onto the cell surface; (ii) followed by diffusion through the cell wall; (iii) then binding to the

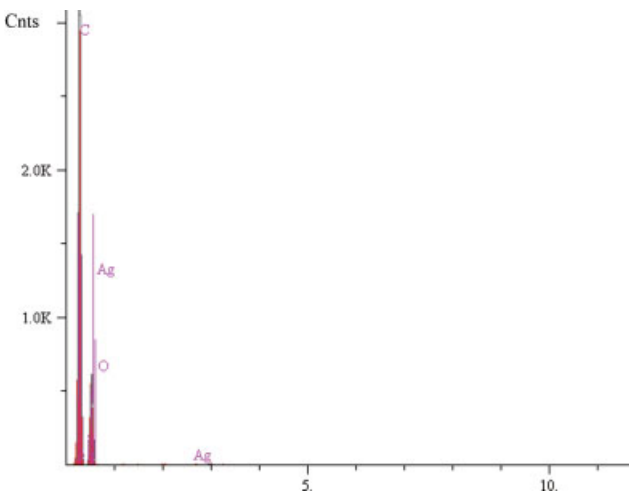


Figure 11 EDX spectrum of MesoSilver–dendrimer composite treated-fabric. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE II
Antimicrobial Test Data Against *Staphylococcus aureus*

Type of microbial agent added	Growth under the sample	Zone of inhibition (mm)
MesoSilver added without dendrimer	NO	1.21
Modified-dendrimer with ammonium functionality	NO	12
$AgNO_3$: dendrimer = 15 : 1	NO	2.6
$AgNO_3$: dendrimer = 32 : 1	NO	2.7
MesoSilver : dendrimer = 1 : 1	NO	1.0
MesoSilver : dendrimer = 3 : 1	NO	2.65
Untreated-fabric	Yes	0

cytoplasmic membranes causing disruption of membranes; (iv) that release cytoplasmic constituents such as DNA, RNA, and K+ followed by the death of a cell. It has been shown that counter ions associated with these ammonium salts have greater influence in the activity of biocides by changing the charge density around the *N* in the salt. It has also been demonstrated that charge density is higher when Cl^{-1} is used as counter ion. Therefore, we used Cl^{-1} as a counter ion when we prepared our quaternary ammonium salt of PAMAM dendrimer.[24]

We used only one type of organism to assess antibacterial activities. Several studies[32–34] with silver nanoparticles and with different organisms have shown that silver is an effective antimicrobial agent against several organisms. However, dispersing these agents with dendrimers provide more effective biocidal activities on large fabric surface area because a large number of antibacterial agents can be provided by dendrimers. The antibacterial activities of silver ions is associated with their interactions with proteins, specifically at thiol (sulfydryl,-SH) groups, and are believed to bind protein molecules together by forming bridges along them. The proteins often behave like enzymes; the cellular metabolism is inhibited, hence causing the destruction of microorganisms.[32,35,36] Silver nanoparticles kill all types of fungal infections, bacteria, and viruses, including antibiotic resistant strains. In our future study, other organisms will be used to investigate the effectiveness of modified dendrimers and dendrimer–silver complexes in biocidal activities. A simulated commercial type wash durability study will also be included.

CONCLUSIONS

Modified dendrimers with ammonium functionalities and silver-dendrimers complexes exhibited antibacterial efficacy against *S. aureus* when applied onto the Nylon/Cotton blend fabric. Silver–dendrimer complexes were prepared with $AgNO_3$ and with MesoSilver solutions. Fabrics treated with dendrimers with ammonium functionalities were found to have a high zone of inhibition with a 12 mm wide zone. This may be attributed to the migratory property, of the ammonium functionalities, to diffuse into the agar. Both the silver–dendrimers complexes showed effective biocidal activities where no bacterial growth was observed on the treated-fabric surface when the fabric specimens were exposed to the bacterial lawn and incubated. The zone of inhibition, however, was relatively small than that of dendrimers with ammonium functionalities. The visual assessment of the SEM images and EDX studies of the fabric treated with the silver–dendrimers complex showed an adequate presence of silver particles. Both FTIR and ^{13}NMR studies also confirmed the conversion of PAMAM (G3) into quaternary ammonium functionalized dendrimers.

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