Journal of Applied Polymer Science

Electrospun Nylon 6 Nanofibers Incorporated with 2-Substituted *N*-Alkylimidazoles and Their Silver(I) Complexes for Antibacterial Applications

Phumelele Kleyi,[1] Carminita L. Frost,[2] Zenixole R. Tshentu,[1] Nelson Torto[1]

[1]Department of Chemistry, Rhodes University, Grahamstown, South Africa

[2]Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, Port Elizabeth, South Africa

Correspondence to: N. Torto (E-mail: n.torto@ru.ac.za)

ABSTRACT: The article presents the incorporation of biocides [2-substituted *N*-alkylimidazoles and their silver(I) complexes] into electrospun nylon 6 nanofibers for application as antimicrobial materials. The electrospun nylon 6/biocides nanofiber composites were characterized by IR spectroscopy (ATR-FTIR) and scanning electron microscopy (SEM-EDX). The antimicrobial activity of the electrospun nylon 6/biocides nanofiber composites was evaluated against *Escherichia coli*, *Staphylococcus aureus,* and *Bacillus subtilis* subsp. *spizizenii* using the disk diffusion method, the American Association for Textile Chemists and Colorists test method 100-2004 and the dynamic shake flask method (American Society for Testing and Materials E2149-10). The electrospun nylon 6 nanofibers incorporated with 2-substituted *N*-alkylimidazoles displayed moderate to excellent levels of growth reduction against *S. aureus* (73.2–99.8%). For the electrospun nylon 6 nanofibers incorporated with silver(I) complexes, the levels of growth reduction were >99.99%, for both *E. coli* and *S. aureus*, after the antimicrobial activity evaluation using the shake flask method. The study demonstrated that the electrospun nanofibers, fabricated using the incorporation strategy, have the potential to be used as attractive antimicrobial materials.
 J. Appl. Polym. Sci. **2014**, *131*, 39783.

KEYWORDS: electrospinning; biomaterials; grafting; nanoparticles; nanowires and nanocrystals

Received 9 May 2013; accepted 17 July 2013

DOI: 10.1002/app.39783

INTRODUCTION

The emergence of new pathogenic micro-organisms necessitates more research to develop materials that possess a broader spectrum of antimicrobial properties. The materials are particularly important for the eradication of pathogenic micro-organisms in clinical applications (wound dressing), food industry, and water treatment. The growth of pathogenic micro-organisms on wounds, in food or in water could cause serious infections to humans. For example, *Escherichia coli* O157:H7 is a Gram-negative bacterium that is usually associated with food poisoning, diarrhea, and haemolytic uremic syndrome.[1] *Staphylococcus aureus* is a Gram-positive bacterium that is usually associated with skin infections and in some cases causes food poisoning.[2] The diseases caused by pathogenic micro-organism contamination have traditionally been treated using organic or inorganic molecules that possess antimicrobial properties.

Azoles as well as silver and its salts are amongst a large range of molecules and metals with well documented antimicrobial properties.[3–6] The antimicrobial properties of azoles could be enhanced by a simple derivatization, by introducing an alkyl chain at the 1-position.[7,8] It was demonstrated that the antimicrobial activity of imidazole increased with increasing alkyl chain length.[7] The antimicrobial properties of the alkylated imidazoles could be further improved by introducing a substituent with a low pK_a at the 2-position.[9] Another strategy used for the enhancement of the antimicrobial properties of azoles was the complexation with metal ions that have antimicrobial properties such as silver(I) ions. Silver and its salts has been used as antimicrobial agents for many centuries as it possesses the most superior properties among all metals with antimicrobial activity.[10–13] There are numerous studies that have demonstrated the attractive antimicrobial properties of silver(I)-imidazole complexes.[14–16] Even though silver(I)-imidazole complexes possess broad spectrum antimicrobial properties the activity is usually entirely due to silver(I) ions.[17–19] Our group has recently reported silver(I)-imidazole complexes with broad spectrum antimicrobial properties contributed by both silver(I) ions and the imidazole moiety.[20]

Additional Supporting Information may be found in the online version of this article.

In recent times, polymer nanofibers fabricated by the electrospinning process have received much attention for use as antimicrobial materials.[21–25] Electrospinning is a simple technique for the fabrication of nanofibers, from a polymer melt or solution, under the influence of electric forces (charges). Several polymeric nanofiber materials with antimicrobial properties were reported and the antimicrobial properties of the electrospun nanofiber materials were evaluated against various microorganisms.[26–28] Most polymers (e.g. nylon 6, polystyrene) have no inherent antimicrobial properties; thus the antimicrobial properties are induced by the incorporation of molecules that have antimicrobial properties. Electrospun nanofibers doped with silver nanoparticles have been fabricated and their antibacterial properties were evaluated.[29–33] The electrospun chitosan/poly(vinyl alcohol) nanofiber blend incorporated with silver nanoparticles displayed high antibacterial activity against a Gram-negative bacterium (*E. coli*).[32] In contrast, silver nanoparticle doped electrospun carboxyethylchitosan nanofibers showed activity against a Gram-positive bacterium (*S. aureus*).[33] Electrospun nylon 6 nanofibers doped with silver nanoparticles have also displayed excellent antibacterial properties against both Gram-negative and Gram-positive bacteria.[29–31] To the best of the authors' knowledge, there has not been any work reported on the antimicrobial activity of electrospun nylon 6 nanofibers incorporated with 2-substituted *N*-alkylimidazoles and their silver complexes. Therefore, the study presents the electrospun nylon 6 nanofibers incorporated with 2-substituted *N*-alkylimidazoles and their silver(I) complexes for use as antimicrobial materials.

EXPERIMENTAL

Reagents and Instrumentation

Nylon 6 ($M_w = 11200$ Da) was obtained from Sigma Aldrich (Milwaukee). Formic acid (85%) and acetic acid (95%), Meuller-Hinton and Nutrient (agar/broth) were obtained from Merck Chemicals (Johannesburg, SA) and were used as received. *E. coli* (ATCC 8793), *S. aureus* (ATCC 6538), and *B. subtilis* subsp. *spizizenii* (ATCC 6633) were obtained from Microbiologics (Minnesota). Metronidazole discs (50 μg), and blank discs (6.5 mm) were sourced from Davies Diagnostics (Johannesburg, SA). Metronidazole (for preparing 100 μg discs) was purchased from Changzhou Longcheng Medicine Raw Material Co. (Changzhou City, Jiangsu, China), and ketoconazole was purchased from Oman Chemicals and Pharmaceuticals (Al Buraimi, Sultanate of Oman).

Infrared spectra were acquired with a Perkin Elmer Spectrum 400 FT-IR spectrometer (Massachusetts). The morphology of electrospun nylon 6 nanofibers was studied using a Tescan (TS5136ML) Scanning Electron Microscope (Brno, Czech Republic) operating at an accelerated voltage of 20 kV after gold sputter coating. UV-visible absorption spectrum for AgNPs was recorded on a Perkin Elmer Lambda 25 UV/Vis spectrometer using quartz cuvettes with an optical path length of 1 cm. The electrospun nylon 6 nanofibers embedded silver nanoparticles were characterized using an Oxford Instruments INCAPentaFET x3 Energy dispersive spectroscope (Bristol, UK) fitted with INCA Analyzer software. The flow rate of the polymer solution was controlled using a NE-300 Just Infusion^{TM} syringe pump (New York). Viable bacterial colonies were enumerated using a synbiosis aCOLade colony counter (Cambridge, UK). For the cytotoxicity experiments, absorbance was measured using a Biotek Powerwave XS Spectrophotometer (Winooski) and the results were analyzed using GraphPad Prism 5 software.

Fabrication of Electrospun Nylon 6/Biocides Nanofiber Composites

The polymer solution for electrospinning was prepared by dissolving nylon 6 (1.6 g, 16% [w/v]) and the biocide (0.08 g, 5% [w/w]) in 10 mL mixture of $HCOOH/CH_3COOH$ (1:1). All electrospinning parameters were optimized at ambient conditions as follows; the flow rate (0.75 mL/h), the applied voltage (+22.5 kV, −5 kV), the tip-to-collector distance (8 cm). The flow rate of the polymer solution was controlled using a digital pump. For the electrospinning of nylon 6 nanofibers incorporated with silver(I) complexes and silver nanoparticles, the applied voltage was adjusted slightly. The silver nanoparticles were prepared by *in situ* reduction of $AgNO_3$ in a nylon 6 solution without the addition of a reducing agent.[34] Formic acid, one of the solvents used for the dissolution of nylon 6, is known to be capable of reducing Ag(I)-Ag(0).

Antimicrobial Activity Evaluation

Disk Diffusion Method. The antimicrobial activity of the electrospun nylon 6/biocides nanofiber composites was evaluated against Gram-negative (*E. coli* ATCC 8793) and Gram-positive (*S. aureus* ATCC 6538 and *B. subtilis,* subsp. *spizizenii* ATCC 6633) bacterial strains using the disk diffusion, American Association for Textile Chemists and Colorists (AATCC) test method 100-2004 and the dynamic shake flask method (ASTM E2149-10). The bacterial culture suspensions were prepared by suspending the respective bacterial colonies in Millipore water. A 0.5 McFarland standard ($OD_{625} = 0.08–0.13$, 1.5×10^8 CFU/mL) was used to match the turbidity of the bacterial culture suspensions. To determine the antimicrobial activity, disks (7.0 ± 0.1 mm) were cut from the electrospun nylon 6/biocides nanofiber composites, and placed onto Mueller-Hinton agar plates streaked with the various bacteria. The plates were incubated at 37°C for 18 h after which the zones of clearance were measured. Pristine electrospun nylon 6 nanofibers were used as a negative control.

AATCC Test Method 100-2004. The antimicrobial activity of electrospun nylon 6 nanofibers was investigated using a modified version of the AATCC Test Method 100-2004.[35] *E. coli* and *S. aureus* were used as model challenge micro-organisms. A diluted bacterial suspension with approximately 1×10^8 CFU/mL concentration was used, and 500 μL of this suspension was loaded onto the electrospun nylon 6 nanofiber swatches (approximately 4.8 cm in diameter) in the presence of a non-ionic wetting agent (Triton X-100). The inoculum on the surface was then carefully covered with another identical nylon 6 nanofiber in a sterilized glass jar. After incubation for 24 h contact time, 0.02 *N* sodium thiosulfate was added in excess to quench the biological growth. The mixture was then vortexed vigorously for 2 min. An aliquot of the solution was serially diluted and plated onto nutrient agar plates. The same

procedure was applied to electrospun nylon 6 nanofibers with no additives as a negative control. Viable bacterial colonies on the agar plates were counted after incubation at 37°C for 48 h. The reduction rate in the number of bacteria was calculated using Eq 1:

$$R(\%)=\frac{N_0-N_t}{N_0}\times 100 \quad (1)$$

where R is the reduction rate, N_t is the number of bacteria recovered from the inoculated electrospun nylon 6 nanofibers over 24 h of contact time, and N_o is the number of bacteria recovered from the inoculated electrospun nylon 6 nanofibers at zero contact time.

Dynamic Shake Flask Method (ASTM E2149-10). The antimicrobial activity of electrospun nylon 6/biocides nanofiber composites was evaluated using the dynamic shake flask test method (American Society for Testing and Materials [ASTM] E2149).[36] A working suspension was prepared by diluting, with a sterile 3 m*M* phosphate buffer (pH 7.2 ± 0.1), a 24 h culture to an optical density (absorbance) of 0.28 ± 0.02 at 475 nm (1.5–30 × 10^8 CFU/mL). Further appropriate dilution using a sterile phosphate buffer gave a final concentration of 1.5–30 × 10^5 CFU/mL. Electrospun nylon 6 nanofiber composites (0.2–0.3 g) were placed into flasks-containing 20 mL of the working suspension. The flasks were incubated with continuous shaking at 37°C for 1 h. After serial dilutions using the phosphate buffer, the bacterial suspensions (0.1 mL) were plated on nutrient agar. The inoculated plates were incubated at 37°C for 24 h and surviving bacterial cells counted using a colony counter. The percentage reduction of the microorganisms after contact with the test nylon 6/biocides nanofiber composites was compared to the number of bacterial cells surviving in an untreated suspension as the control. Equation 1 was used to calculate the percentage reduction of microbial growth.

Cytotoxicity Studies

Chang liver cells were seeded in 10 cm diameter culture dishes, maintained in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum. Cells were incubated in a humidified atmosphere-containing 5% CO_2 at 37°C. Chang liver cells were seeded at a density of 6000 cells/200 μL in 96 well microplates. After 24 h, the medium was removed from the cells and replaced with RPMI medium-containing each test compound ranging from a concentration of 100–0.098 μg/mL. Viable cells were determined using the 4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide (MTT) assay.[37] After the various treatments, the medium in all the wells was aspirated, replaced with 100 μL of 0.5 mg/mL MTT, and incubated at 37°C for 1 h, after which the MTT was removed through aspiration. The formazan crystals were solubilized by the addition of 100 μL DMSO and the absorbance measured at 570 nm. All results were analyzed using the Graphpad Prism 5 data analysis program.

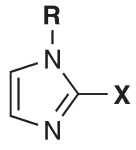
RESULTS AND DISCUSSION

Fabrication and Characterization of Electrospun Nylon 6/Biocides Nanofiber Composites

Incorporation of 2-Substituted *N*-Alkylimidazole into Electrospun Nylon 6 Nanofibers. The *N*-alkylimidazole derivatives (alkyl=octyl and decyl)-containing various substituent groups (carboxaldehyde [CHO], alcohol [CH_2OH], and carboxylic acid [COOH]) at the 2-position of the imidazole moiety were synthesized as reported previously.[9] The synthesis methods and the characterization data for the N-alkylimidazole derivatives can be obtain in the Supplementary information section. The first step was the synthesis of *N*-alkylimidazoles which was carried out by the reaction of imidazole and alkylbromides in the presence of potassium hydroxide. The reaction of *N*-alkylimidazoles and *n*-butyllithium at −78°C (dry ice/acetone slurry) followed by the addition of dry DMF gave the desired *N*-alkylimidazole-2-carboxaldehydes in moderate to excellent yields.[9] *N*-alkylimidazole-2-methanols were obtained by the reduction of *N*-alkylimidazole-2-carboxaldehydes with sodium borohydride at 0°C in methanol.[9] The novel synthesis of *N*-alkylimidazole-2-carboxylic acids was carried out by the oxidation of *N*-alkylimidazole-2-carboxaldehydes with 30% hydrogen peroxide in water.[9] This "green" method was carried out at room temperature and it produced water as the only by-product and thus is an environmentally-friendly alternative to the current metal-catalyzed oxidation methods. Figure 1 illustrates the general structure of the 2-substituted *N*-alkylimidazoles. Nylon 6 was chosen as a solid support material, to host the biocides, because of its biocompatibility, biodegradability, mechanical stability, electrospinnability, and insolubility in water.[38–40]

The morphology of the electrospun nylon 6 nanofiber composites was characterized using scanning electron microscopy (SEM). SEM micrographs (Figure 2) showed that smooth or beadless nanofibers with uniform diameters were obtained under the electrospinning conditions. The diameters ranges of the electrospun nanofibers incorporated with 2-substituted *N*-alkylimidazoles were *N*-octylimidazole-2-carboxaldehyde (39–155 nm), 2-hydroxymethyl-*N*-octylimidazole (59–150 nm), and *N*-octylimidazole-2-carboxylic acid (72–146 nm) using the ImageJ software.

The electrospun nylon 6 nanofiber composites were also characterized using ATR-FTIR to ascertain the presence of the compounds within the electrospun nanofiber matrix (Figure 2). Two bands were observed in the region 1200–1000 cm^{-1} and one band in the region 800–650 cm^{-1} in the electrospun nylon 6 nanofiber composite which coincided with bands observed in 2-hydroxymethyl-*N*-octylimidazole (Figure 3). The bands were a confirmation that the compound was successfully incorporated



R	X = CHO	X = CH_2OH	X = COOH
octyl	octImCHO	octImCH_2OH	octImCOOH
decyl	decImCHO	decImCH_2OH	decImCOOH

Figure 1. The general structure of the 2-susbtituted *N*-alkylimidazole derivatives.

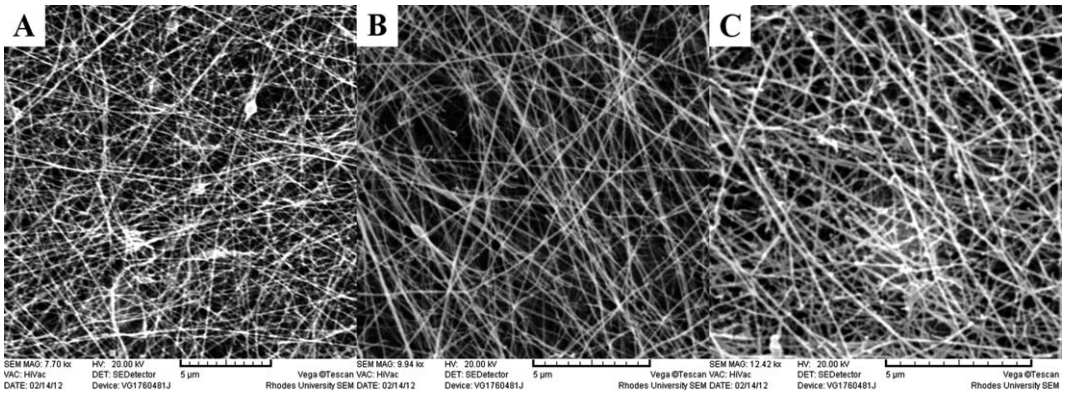


Figure 2. Electrospun nanofibers incorporated with; (A): *N*-octylimidazole-2-carboxaldehyde (39–155 nm), (B): 2-hydroxymethyl-*N*-octylimidazole (59–150 nm), and (C): *N*-octylimidazole-2-carboxylic acid (72–145 nm) and their diameter ranges.

into the electrospun nylon 6 nanofibers. The intensities of the bands were however very low and the effect was expected considering the mass of the compounds (5% w/w) used with respect to the mass of polymer (nylon 6). The intensities of the bands associated with the incorporated compounds were believed to have been suppressed by those of the polymer (nylon 6).

Incorporation of Silver(I) Complexes into Electrospun Nylon 6 Nanofibers. The silver(I) complexes-containing 2-hydroxymethyl-*N*-alkylimidazoles (Figure 4) were also synthesized as previously reported.[20] Electrospun nylon 6 nanofibers incorporated with Ag(I) complexes-containing 2-hydroxymethyl-*N*-alkylimidazoles (R═octyl and decyl) were fabricated using the optimized conditions as described for the electrospun nylon 6 nanofibers incorporated with 2-substituted *N*-alkylimidazoles. However, slight adjustments of the applied voltage were effected so as to obtain a stable polymer jet, that is, the applied voltage was increased by +0.5 to +1 kV from (+22.5 kV, −5 kV) while the negative voltage was kept constant. The morphology of the electrospun nylon 6 nanofibers was characterized using SEM while the presence of the Ag(I) complexes after electrospinning was ascertained using ATR-FTIR spectroscopy.

SEM micrographs revealed that the nanofibers obtained had uniform diameters with A (52–141 nm) and B (74–187 nm) using ImageJ software (Figure 5). It was very difficult to identify bands belonging to the silver(I) complexes used for the fabrication of the antimicrobial nylon 6 nanofibers. The challenge was attributed to the suppression of the bands belonging to the silver(I) complexes by the nylon 6 bands due to the relative mass (5% w/w) of the compounds that was incorporated into the nanofibers. Nonetheless, a low intensity band could be identified in the region 1000–1200 cm^{-1} which corresponded to a band from the spectrum of the silver(I) complexes as shown in Figure 6. The band was taken as a confirmation that the silver(I) complexes were successfully incorporated into the electrospun nylon 6 nanofibers.

The incorporation of the silver(I) complexes into electrospun nylon 6 nanofibers was further investigated using SEM-EDS as shown in Figure 7. The SEM-EDS spectrum displayed a silver signal which confirmed the presence of the silver(I) complexes within the electrospun nylon 6 nanofiber matrix.

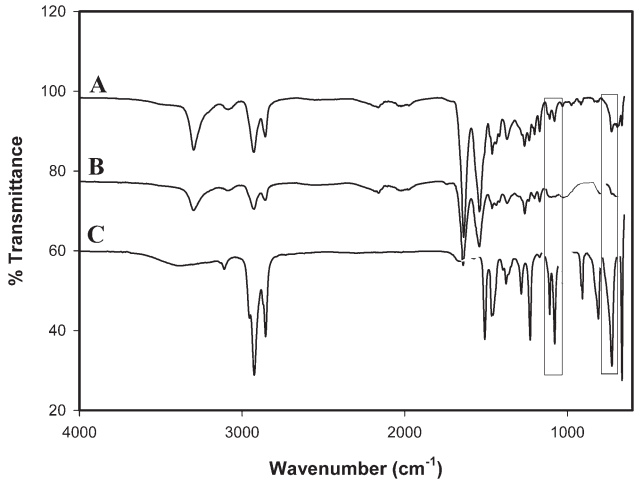


Figure 3. ATR-FTIR spectra of (A): nylon 6 nanofiber/octImCH$_2$OH composite, (B): nylon 6, and (C) 2-hydroxymethyl-*N*-octylimidazole (octImCH$_2$OH).

Incorporation of Silver Nanoparticles into Electrospun Nylon 6 Nanofibers. Electrospun nylon 6 nanofibers incorporated with silver nanoparticles (AgNPs) were also fabricated for comparison with antimicrobial activity of electrospun nanofibers

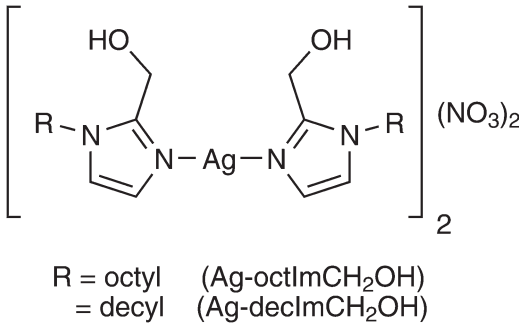


Figure 4. The structure of the silver(I) complexes-containing 2-hydroxymethyl-*N*-alkylimidazole.

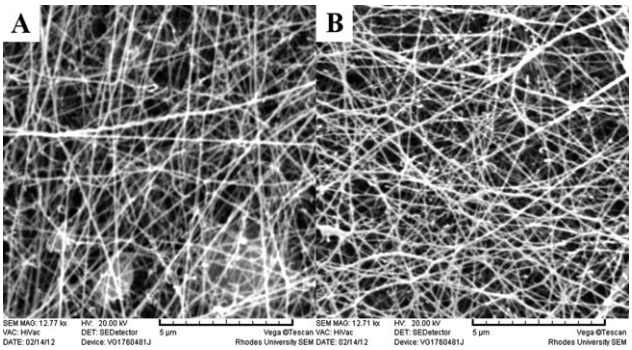


Figure 5. SEM micrographs of electrospun nylon 6 nanofibers incorporated with silver(I)-imidazole complexes; (A) nylon 6/silver(I) complex (R═octyl) nanofibers (52–141 nm) and (B) nylon 6/silver(I) complex (R═decyl) nanofibers (74–187 nm) and their diameter ranges.

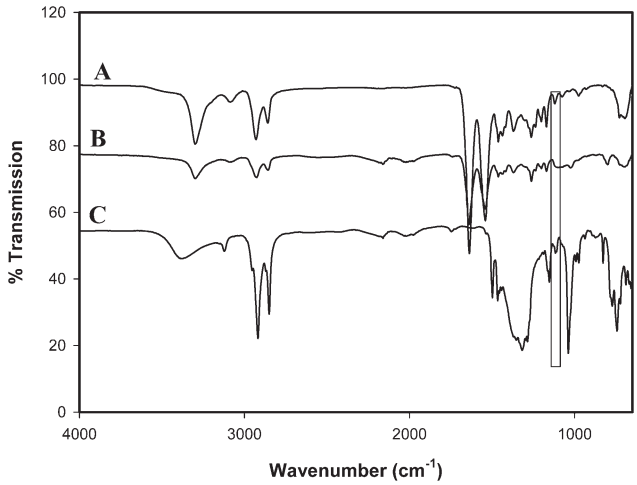


Figure 6. ATR-FTIR spectra of (A) nylon 6 nanofibers; (B) nylon 6/silver(I)-imidazole nanofiber composite, and (C) silver(I) complex (R═decyl).

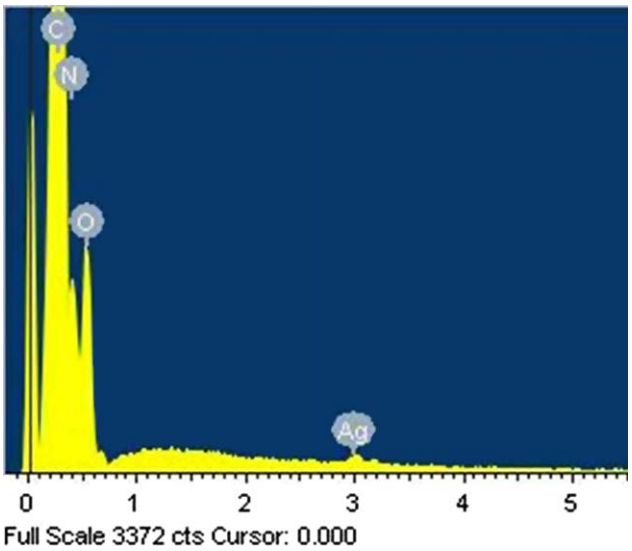


Figure 7. SEM-EDS spectrum (eV) of electrospun nylon 6 nanofibers incorporated with Ag-decImCH$_2$OH. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

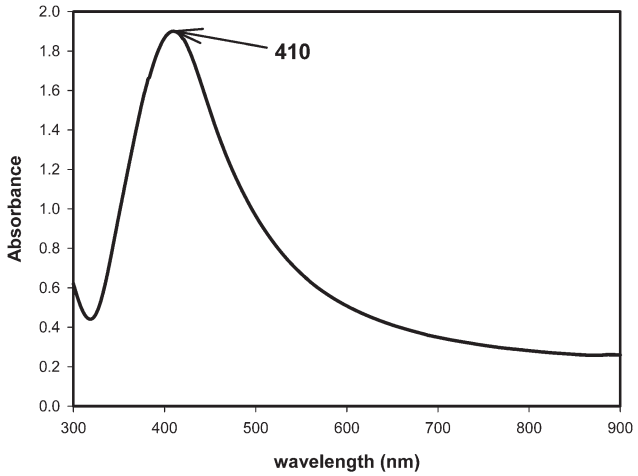


Figure 8. UV-Vis spectrum of showing the surface plasmon resonance of the AgNPs in nylon 6 solution.

incorporated with silver(I) complexes. Figure 8 presents a UV spectrum of AgNPs in a nylon 6 solution before electrospinning. The UV spectrum indicated that the AgNPs experienced a sharp surface plasmon resonance at 410 nm; indicative of spherically-shaped nanoparticles with a narrow size distribution.[41]

The presence of the AgNPs in the electrospun nylon 6 nanofibers matrix was also confirmed using SEM-EDS. Figure 9 depicts an SEM-EDS spectrum which illustrated the presence of the AgNPs within the electrospun nylon 6 nanofibers. The relative intensities of the silver signals were lower compared to the intensities of the elements for nylon 6 due to the mass of $AgNO_3$ (5% w/w) used for the fabrication of electrospun nanofiber composites. Once incorporation of the 2-substituted *N*-alkylimidazoles, their silver(I) complexes and nanoparticles was ascertained, the antimicrobial activity of the resultant electrospun nylon 6/biocides nanofiber composites was evaluated.

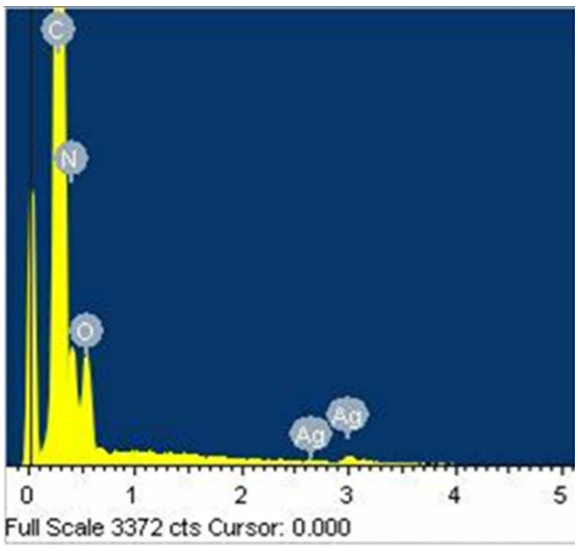


Figure 9. SEM-EDS spectrum (eV) of electrospun nylon 6 nanofibers incorporated with AgNPs. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

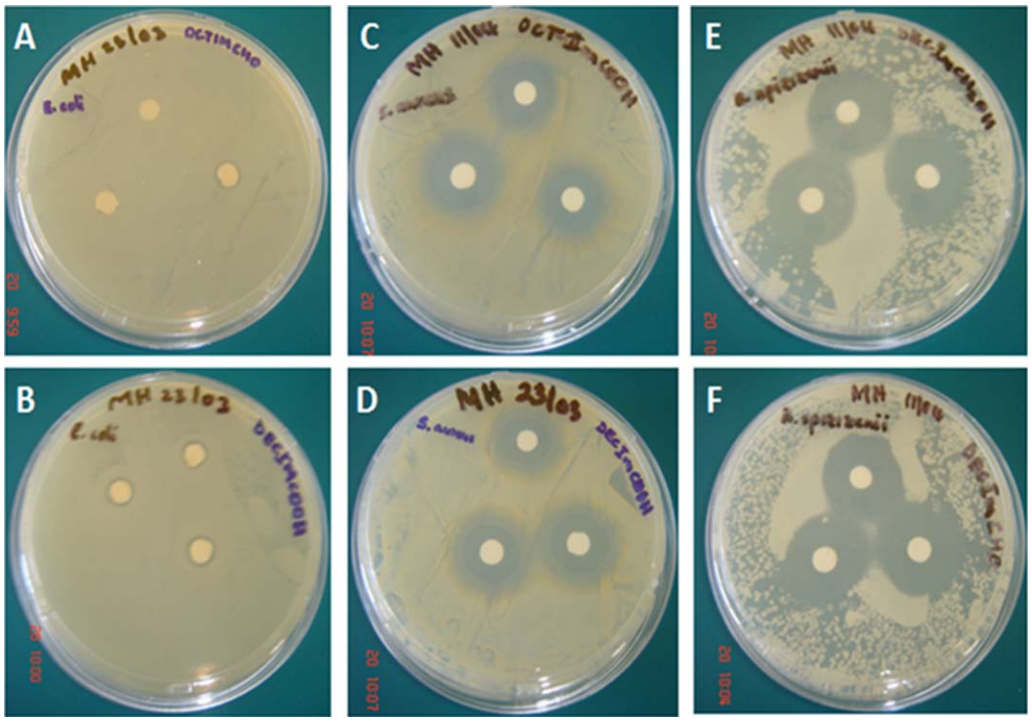


Figure 10. Zones of inhibition illustrating antimicrobial activity for electrospun nylon 6 nanofiber composites-containing 2-substituted *N*-alkylimidazoles towards *E. coli* (A & B), *S. aureus* (C & D), and *B. subtilis* subsp, *spizizenii* (E & F). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Antimicrobial Activity Evaluation

The disk diffusion method was used to evaluate the antibacterial properties electrospun nylon 6/biocides nanofiber composites. Disks (diameter = 7.0 ± 0.1 mm) were cut from the electrospun nylon 6 nanofiber composites and used for antimicrobial testing. Disk diffusion results showed that the 2-substituted *N*-alkylimidazoles maintained the antimicrobial properties even after incorporation into the polymer nanofibers and the activity was comparable to when the 2-*N*-alkylimidazoles were tested separately.[9] It was also observed that the antimicrobial activity remained predominantly against Gram-positive bacteria (*S. aureus* and *B. subtilis* subsp. *spizizenii*) with the latter being the most susceptible (Figure 10, Table I). In addition, the electrospun nylon 6 nanofiber composites showed poor antimicrobial activity towards *E. coli*. The advantage of using electrospun nanofibers incorporated with biocides was attributed to the attractive capability of controlled release of biocides, over long periods, due to their highly porous nature.[42–45] Once the antimicrobial activity of the electrospun nylon 6 nanofiber composites was established, the AATCC test method 100 suitable for evaluating the antimicrobial properties of treated fabrics, was used to investigate the reduction of microbial growth by the electrospun nylon 6 nanofiber composites.

The American Association of Textile Chemists and Colorists (AATCC) Test Method 100-2004[35] was used to investigate the antibacterial activity of the electrospun nylon 6 nanofibers incorporated with 2-substituted *N*-alkylimidazoles. In the AATCC test method, the antibacterial activity of electrospun nanofibers was depicted by the percentage reduction of the microbial growth. The percentage reduction of microbial growth was calculated using Eq 1.

Table I. Diameters of Zones of Clearance for the Electrospun Nylon 6 Nanofiber-Containing 2-Substituted *N*-Alkylimidazoles

	E. coli	*S. aureus*	*B. subtilis* subsp. *spizizenii*
Nanofibers composite	Diameter (mm)	Diameter (mm)	Diameter (mm)
Nylon 6-octImCHO	7.2 (±0.3)	12.7 (±0.3)	29.7 (±0.6)
Nylon 6-decImCHO	7.0 (±0.1)	11.8 (±1.0)	22.7 (±0.6)
Nylon 6-octImCH_2OH	8.3 (±0.6)	12.8 (±1.2)	22.0 (±1.7)
Nylon 6-decImCH_2OH	7.7 (±0.6)	12.2 (±1.0)	24.0 (±1.7)
Nylon 6-octImCOOH	7.3 (±0.6)	16.7 (±0.6)	23.3 (±2.3)
Nylon 6-decImCOOH	8.0 (±0.1)	15.7 (±0.8)	28.7 (±0.6)

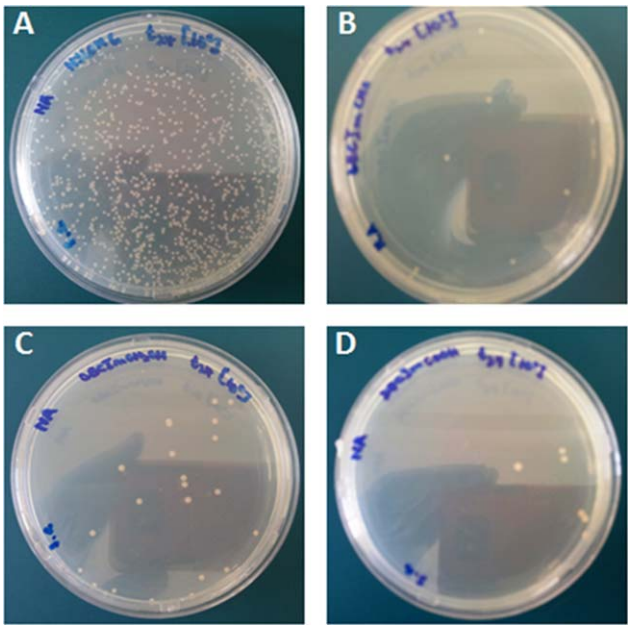


Figure 11. The growth of *S. aureus* after 24 h contact time with antimicrobial nanofibers. (A) nylon 6 nanofibers; (B) nylon 6-decImCHO nanofibers; (C) nylon 6-decImCH_2OH nanofibers, and (D) nylon 6-decImCOOH nanofibers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The electrospun nylon 6 nanofiber composites were first cut into swatches (diameter = 4.8 ± 0.1 cm) which were used for conducting the experiments. Since the disk diffusion results showed that the electrospun nylon 6 nanofiber composites were predominantly active against Gram-positive bacteria (*S. aureus* and *B. subtilis* subsp. *spizizenii*), only one strain was chosen for the AATCC test method. *Staphylococcus aureus* was used to perform the experiments since it was the easier of the two Gram-positive strains to work with. The percentage reduction of bacterial growth against *S. aureus* ranged between 73.2 and 99.8% for the 2-substituted *N*-alkylimidazoles, with electrospun nylon 6 nanofibers-containing the carboxylic acid derivatives displaying the largest growth reduction. The antibacterial properties were comparable to those reported by Jeong et al.[22] for the electrospun polyurethane cationomer nanofibers (>99.9%). Figure 11 illustrates the reduction of bacterial growth due to electrospun nylon 6 nanofiber composites in comparison to pristine nylon 6 nanofibers.

The antimicrobial evaluation results showed that the electrospun nylon 6 nanofibers incorporated 2-substituted *N*-alkylimidazoles displayed excellent antimicrobial activity only against Gram-positive bacteria. Thus, the 2-substituted *N*-alkylimidazoles were then used to synthesize silver(I) complexes, in an attempt to effect broader spectrum antimicrobial properties. The antimicrobial activity of electrospun nylon 6 nanofibers incorporated with silver(I) complexes-containing 2-hydroxymethyl-*N*-alkylimidazoles (R=octyl and decyl) with long chains was investigated using the disk diffusion and ASTM E2149-10 methods. The 2-substituted *N*-alkylimidazoles-containing the carboxaldehyde and the carboxylic acid groups could not be used for the synthesis of the silver(I) complexes due to reduction of silver ions by carbonyl compounds.

The disk diffusion method showed that the electrospun nylon 6/silver(I)-imidazole nanofiber composites possessed broad spectrum antimicrobial properties (Table II). The antimicrobial activity of the electrospun nylon 6 nanofiber composites was the highest against Gram-positive bacteria (*S. aureus* and *B. subtilis* subsp. *spizizenii*) as was observed for the 2-substituted *N*-alkylimidazoles when tested separately.[20] Furthermore, the antimicrobial activity of the electrospun nylon 6 nanofibers incorporated with silver(I) complexes was compared with that of electrospun nylon 6 nanofibers incorporated with silver nanoparticles (AgNPs).

The electrospun nylon 6 nanofibers incorporated with AgNPs exhibited poor antimicrobial activity in comparison to the electrospun nylon 6 nanofibers incorporated with silver(I) complexes. The observation was, however, not surprising since silver(0) does not have antimicrobial activity unless it is oxidized to silver(I) by moisture.[46] The poor antimicrobial activity of the nanoparticles suggested that there was not sufficient moisture to oxidize Ag(0)-Ag(I) in the culture medium, resulting in lower concentration of Ag(I) ions. Further evaluation of the antimicrobial activity of electrospun nylon 6 nanofibers was performed using the ASTM E2149 method 10.[36]

The dynamic shake flask testing method (ASTM E2149-10) is an approved method for the evaluation of the antimicrobial activity of immobilized antimicrobial agents.[36] The method is performed under dynamic conditions to allow efficient contact of the micro-organisms with the treated antimicrobial materials. Moreover, the method has also been used to evaluate the antimicrobial activity of electrospun nanofibers incorporated with AgNPs.[34,47] The shake flask method, as with AATCC test method 100, expresses the antimicrobial activity as the percentage reduction of the growth of micro-organisms.

Figure 12 illustrates the results obtained from the antimicrobial activity evaluation of electrospun nylon 6/silver(I)-imidazole nanofiber composites using the dynamic shake flask method. All

Table II. Diameters of the Zones of Clearance ($n = 3$) for the Electrospun Nylon 6 Nanofibers Incorporated with Ag(I) Complexes (R=octyl and decyl)

	E. coli	*S. aureus*	*B. subtilis* subsp. *spizizenii*
Nanofiber composite	Diameter (mm)	Diameter (mm)	Diameter (mm)
Nylon 6-Ag-octImCH_2OH	13.3 (±0.6)	22.3 (±0.6)	24.3 (±1.5)
Nylon 6-Ag-decImCH_2OH	10.7 (±0.6)	15.7 (±0.6)	16.7 (±0.6)
Nylon 6-AgNPs	9.7 (±0.6)	12.0 (±0.0)	11.0 (±0.0)

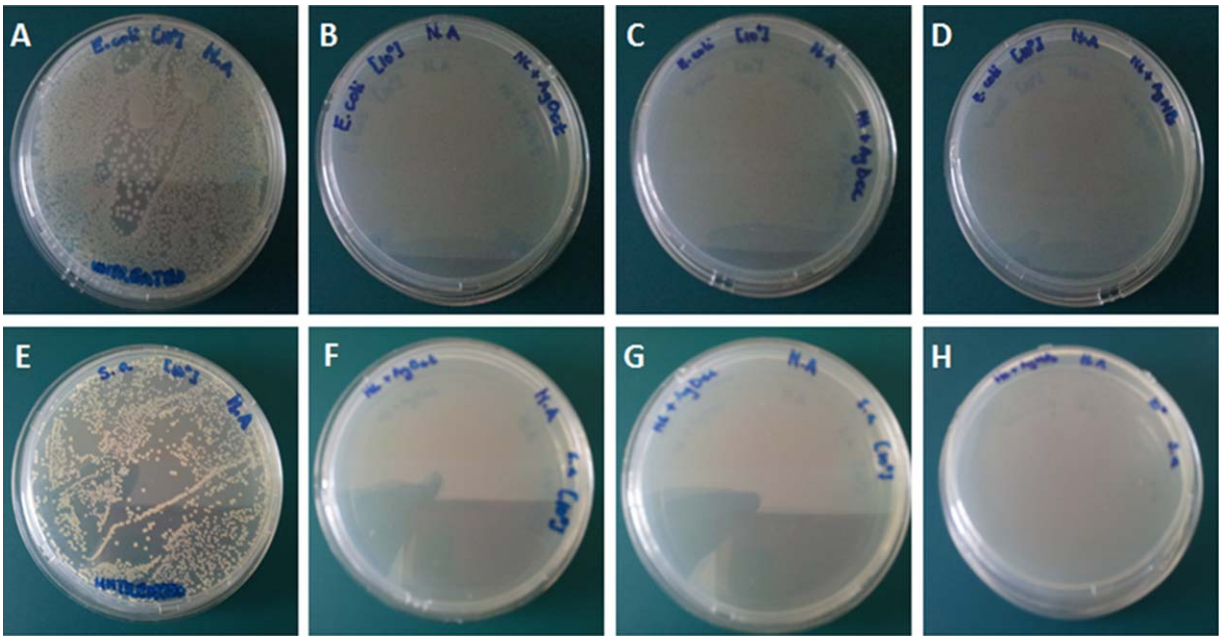


Figure 12. Bacterial growth after contact with antimicrobial nanofibers. *E. coli* (A–D) and *S. aureus* (E–H). A and E represent the bacterial growth for the untreated suspensions ($t = 0$) while B–D and F–H represent the bacterial growth at $t = 1$ h. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the electrospun nylon 6 nanofibers composites, incorporated with silver(I) complexes and AgNPs, that were evaluated displayed very high percentage reduction of microbial growth (>99.99%) for both *E. coli* and *S. aureus*. Similar antibacterial activities have been reported by Melaiye and co-workers for the electrospun tecophilic nanofibers incorporated with silver(I)-imidazole cyclophane *gem*-diol complexes.[48] However, higher quantities of the silver(I)-imidazole complexes (25–75%) were used in their study compared to this study (5%). The results obtained from the shake flask test method showed that electrospun nylon 6 nanofibers incorporated with silver(I)-imidazole complexes retained the antimicrobial activity after incorporation into electrospun nylon 6 nanofibers.

Cytotoxicity Studies

The cytotoxic effects of 2-substituted *N*-alkylimidazoles, silver(I) complexes and electrospun nylon 6 nanofibers were evaluated on the Chang liver cells. The liver cells were chosen since the liver is the organ where the effects of cytotoxic molecules that enter the human body are manifested. Table III presents the IC_{50} values obtained from the cytotoxicity experiments. The data showed that the electrospun nylon 6 nanofibers incorporated with biocides have lower IC_{50} values compared to pristine electrospun nylon 6 nanofibers. Many studies have reported the cytotoxic effects of imidazole derivatives against various human cell lines.[49–53] Assadieskandar et al.[53] considered their imidazole derivatives to be highly cytotoxic against various human cell lines with the following IC_{50} values; HT-29 (0.18 to >50 μM); MCF-7 (0.40–22.90 μM); NIH-3T3 (0.02 to >50 μM), and AGS (0.04–48.43 μM). The IC_{50} values for imidazole derivatives against K562 (140–220 μM) and CEM cells (210–245 μM) were considered to be moderate.[49] In comparison, the IC_{50} values displayed by decImCHO (5.84×10^{-5} μM) and Ag-decImCH$_2$OH (1.87×10^{-5} μM) were significantly lower and could be considered to be potentially cytotoxic to the humans.

Table III. IC_{50} Values for the Tested Compounds and Nanofibers

Test compound or material	IC_{50} (μg/mL and μM)
DecImCHO	13.80 (5.84×10^{-5})
Ag-decImCH$_2$OH	10.91 (1.87×10^{-5})
Nylon 6 nanofibers	33.20[a]

Value in parentheses represents the IC_{50} in μM.
[a]The value represents the mass of solid material per milliliter of suspension.

CONCLUSIONS

The article presented the fabrication of antimicrobial electrospun nylon 6 nanofibers by the incorporation with 2-substituted *N*-alkylimidazoles and their silver(I) complexes. The electrospun nylon 6 nanofibers incorporated with 2-substituted *N*-alkylimidazoles displayed excellent antimicrobial activity against Gram-positive bacteria (*S. aureus* and *B. subtilis* subsp. *spizizenii*) while no activity was observed against Gram-negative bacteria (*E. coli*). However, the electrospun nylon 6 nanofibers incorporated with silver(I)-imidazole complexes showed broad spectrum of antimicrobial properties; that is, they were active against all the test micro-organisms. It was observed that the antimicrobial electrospun nylon 6/biocides composites showed greater possible cytotoxic effects to humans since they displayed

lower IC_{50} values compared to pristine nylon 6 nanofibers. Thus the study demonstrated that the antimicrobial electrospun nanofibers with the potential for use in wound dressings and water disinfection could be fabricated by a simple incorporation of biocides. However, for drinking water disinfection the cytotoxic effects of the electrospun nanofibers, due to the possibility of leaching of the biocides, should be taken into consideration.

ACKNOWLEDGMENTS

The authors would like to thank the Water Research Commission (SA), the International Foundation of Science (Sweden) and the National Research Foundation (SA) for financial assistance.

REFERENCES

1. Besser, R. E. L., S. M.; Weber, J. T.; Doyle, M. P.; Barret, T. J.; Wells, J. G.; Griffin, P. M. *J Am Med Assoc* **1993**, *269*, 2217.
2. Maguire, G. P.; Arthur, A. D.; Boustead, P. J.; Dwyer, B.; Currie, B. *J. Med J Aus* **1996**, *164*, 721.
3. Antolini, M.; Bozzoli, A.; Ghiron, C.; Kennedy, G.; Rossi, T.; Ursini, A. *Bioorg amp; Med Chem Lett* **1999**, *9*, 1023.
4. Bellina, F.; Cauteruccio, S.; Rossi, R. *Tetrahedron* **2007**, *63*, 4571.
5. Freeman, C. D.; Klutman, N. E.; Lamp, K. C. *Drugs* **1997**, *54*, 679.
6. Goker, H.; Ertan, R.; Akgun, H.; Yulug, N. *Archiv der Pharmazie* **1991**, *324*, 283.
7. Khabnadideh, S.; Rezaei, Z.; Khalafi-Nezhad, A.; Bahrinajafi, R.; Mohamadi, R.; Farrokhroz, A. A. *Bioorg Med Chem Lett* **2003**, *13*, 2863.
8. Khan, N.; Soni, L.; Gupta, A.; Wakode, S.; Wagh, R.; Kaskhedikar, S. *Indian J Pharm Sci* **2006**, *68*, 341.
9. Kleyi, P.; Walmsley, R. S.; Gundhla, I. Z.; Walmsley, T. A.; Jauka, T. I.; Dames, J.; Walker, R. B.; Torto, N.; Tshentu, Z. R. *South African J Chem* **2012**, *65*, 231.
10. Klasen, H. *J. Burns* **2000**, *26*, 117.
11. Lansdown, A. B. *J Wound Care* **2002**, *11*, 125.
12. Li, W. R.; Xie, X. B.; Shi, Q. S.; Zeng, H. Y.; Ou-Yang, Y. S.; Chen, Y. B. *Appl Microbiol Biotechnol* **2010**, *85*, 1115.
13. Radheshkumar, C.; Münstedt, H. *React Funct Polym* **2006**, *66*, 780.
14. Patil, S.; Deally, A.; Gleeson, B.; Hackenberg, F.; Müller-Bunz, H.; Paradisi, F.; Tacke, M. *Zeitschrift fur Anorganische und Allgemeine Chemie* **2011**, *637*, 386.
15. Özdemir, İ.; Özcan, E. Ö.; Günal, S.; Gürbüz, N. *Molecules* **2010**, *15*, 2499.
16. Kazachenko, A. S.; Legler, A. V.; Per'yanova, O. V.; Vstavskaya, Y. A. *Pharm Chem J* **2000**, *34*, 257.
17. Rowan, R.; Tallon, T.; Sheahan, A. M.; Curran, R.; McCann, M.; Kavanagh, K.; Devereux, M.; McKee, V. *Polyhedron* **2006**, *25*, 1771.
18. Abuskhuna, S.; Briody, J.; McCann, M.; Devereux, M.; Kavanagh, K.; Fontecha, J. B.; McKee, V. *Polyhedron* **2004**, *23*, 1249.
19. McCann, M.; Coyle, B.; Briody, J.; Bass, F.; O'Gorman, N.; Devereux, M.; Kavanagh, K.; McKee, V. *Polyhedron* **2003**, *22*, 1595.
20. Kleyi, P.; Walmsley, R. S.; Fernandes, M. A.; Torto, N.; Tshentu, Z. R. *Polyhedron* **2012**, *41*, 25.
21. Huyck, R. H.; Hunley, M. T.; Allen Jr, M. H.; Long, T. E.: Antimicrobial nanoscale fibers from electrospinning zwitterionic copolymers. 1st ed., American Chemistry Society, Polymer preprints, Division of Polymer Chemistry. 235th ACS National Meeting, Springg 2008, New Orleans, United States, 6-10 April **2008**, Vol. *49*, p 410.
22. Jeong, E. H.; Yang, J.; Youk, J. H. *Mat Lett* **2007**, *61*, 3991.
23. Sun, X., Zhang, L., Cao, Z., Deng, Y., Liu, L., Fong, H., Sun, Y. *ACS Appl Mat Interfaces* **2010**, *2*, 952.
24. Tan, K.; Obendorf, S. K. *J Memb Sci* **2007**, *305*, 287.
25. Torres-Giner, S.; Ocio, M. J.; Lagaron, J. M. *Carbohyd Polym* **2009**, *77*, 261.
26. Ignatova, M.; Starbova, K.; Markova, N.; Manolova, N.; Rashkov, I. *Carbohyd Res* **2006**, *341*, 2098.
27. Liu, X.; Lin, T.; Fang, J.; Yao, G.; Zhao, H.; Dodson, M.; Wang, X. *J Biomed Mat Res Part A* **2010**, 94A, 499.
28. El-Newehy, M. H.; Al-Deyab, S. S.; Kenawy, E.-R.; Abdel-Megeed, A. *J Nanomat* **2011**, 2011, 1.
29. Dong, H.; Wang, D.; Sun, G.; Hinestroza, J. P. *Chem Mat* **2008**, *20*, 6627.
30. Montazer, M.; Malekzadeh, S. B. *J Polym Res* **2012**, *19*, 9980.
31. Park, S. W.; Bae, H. S.; Xing, Z. C.; Kwon, O. H.; Huh, M. W.; Kang, I. K. *J Appl Polym Sci* **2009**, *112*, 2320.
32. Nguyen, T.; Tae, B.; Park, J. *J Mat Sci* **2011**, *46*, 6528.
33. Penchev, H.; Paneva, D.; Manolova, N.; Rashkov, I. *Carbohyd Res* **2010**, *345*, 2374.
34. Shi, Q.; Vitchuli, N.; Nowak, J.; Noar, J.; Caldwell, J. M.; Breidt, F.; Bourham, M.; McCord, M.; Zhang, X. *J Mat Chem* **2011**, *21*, 10330.
35. Assessment of antibacterial finishes on textile materials: AATCC Test Method 100. **2006**.
36. Standard test method for determining the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions: ASTM E2149-10. **2010**.
37. Mosmann, T. *J Immunol Meth* **1983**, *65*, 55.
38. Pant, H. R.; Bajgai, M. P.; Nam, K. T.; Chu, K. H.; Park, S.-J.; Kim, H. Y. *Mat Lett* **2010**, *64*, 2087.
39. Heikkilä, P.; Harlin, A. *Eur Polym J* **2008**, *44*, 3067.
40. Billmayer, F. W., Ed. Textbook of Polymer science, 3rd ed.; Wiley & Sons: New York, **1984**.
41. Wiley, B. J.; Im, S. H.; Li, Z.-Y.; McLellan, J.; Siekkinen, A.; Xia, Y. *J Phys Chem B* **2006**, *110*, 15666.
42. Gupta, B.; Anjum, N.; Gulrez, S. K. H.; Singh, H. *J Appl Polym Sci* **2007**, *103*, 3534.
43. Agarwal, S.; Wendorff, J. H.; Greiner, A. *Polymer* **2008**, *49*, 5603.
44. Natu, M. V.; de Sousa, H. C.; Gil, M. H. *Intern J Pharma* **2010**, *397*, 50.

45. Cui, W.; Chang, J.; Dalton, P. D.: Electrospun fibers for drug delivery. In Comprehensive Biomaterials; Paul, D., Ed.; Elsevier: Oxford, **2011**; p 445.

46. Lok, C. N.; Ho, C. M.; Chen, R.; He, Q. Y.; Yu, W. Y.; Sun, H.; Tam, P. K. H.; Chiu, J. F.; Che, C. M. *J Biol Inorg Chem* **2007**, *12*, 527.

47. Pant, B.; Pant, H. R.; Pandeya, D. R.; Panthi, G.; Nam, K. T.; Hong, S. T.; Kim, C. S.; Kim, H. Y. *Coll Surf A: Physicochem Eng Asp* **2012**, *395*, 94.

48. Melaiye, A.; Sun, Z.; Hindi, K.; Milsted, A.; Ely, D.; Reneker, D. H.; Tessier, C. A.; Youngs, W. *J. J Am Chem Soc* **2005**, *127*, 2285.

49. Ranganatha, S. R.; Kavitha, C. V.; Vinaya, K.; Prasanna, D. S.; Chandrappa, S.; Raghavan, S.; Rangappa, K. *Arch Pharm Res* **2009**, *32*, 1335.

50. Sharma, D.; Narasimhan, B.; Kumar, P.; Judge, V.; Narang, R.; De Clercq, E.; Balzarini, *J. Eur J Med Chem* **2009**, *44*, 2347.

51. Bhandari, K.; Srinivas, N.; Marrapu, V. K.; Verma, A.; Srivastava, S.; Gupta, S. *Bioorg Med Chem Lett* **2010**, *20*, 291.

52. Abdel-Wahab, B. F.; Awad, G. E. A.; Badria, F. A. *Eur J Med Chem* **2011**, *46*, 1505.

53. Assadieskandar, A.; Amini, M.; Ostad, S. N.; Riazi, G. H.; Cheraghi-Shavi, T.; Shafiei, B.; Shafiee, A. *Bioorg Med Chem* **2013**, *21*, 2703.