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ANTIBACTERIAL CELLULOSE ACETATE MICROFIBERS CONTAINING PYRIDINE DERIVATIVE COMPLEXES

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Abstract. Pyridine (L^1) and 2,4-dimethylpyridine (L^2) halide complexes of the type of $[ML_2X_2]$ were prepared and characterized *via* FT-IR and ^{1}H NMR. The CA microfibers containing complexes were electrospun and investigated *via* FT-IR. The morphologies of the microfibers were investigated *via* FE-SEM. Antibacterial activities of the complexes and the fibers were investigated.

Keywords: electrospinning, microfibers, antibacterial, pyridine complexes, cellulose acetate.

1. Introduction

Infections related with health care (HCAIs) – nosocomial infections – are infections acquired in hospitals and in other health-care facilities and appear within 48 h or more after admission of patients to an acute-care hospital or within 30 days after having received health care [1]. Each year 2.5 million people die or get seriously debilitated due to HCAIs caused by organisms known as hospital microbes, which are reported to be the most frequent adverse event and have affected millions of patients worldwide leading to significant rates of death, morbidity, and economic losses of more than 4-5 billion USD [2]. The nosocomial pathogens that are most widely found and pose risk are bacteria, viruses, and fungal parasites. The resistance that bacteria, which is the most serious family of pathogens and infection burden, have developed against the known antimicrobial agents has become an important problem [3]. Much effort has been devoted to overcoming the problem of antibiotic resistance that caused great economic loss (>$105 billion) and thousands of deaths worldwide [3].

Pyridine derivatives show various biological activities and play essential role in physiological functions. They are frequently used in coordination compounds in the synthesis of medical agents. Among them nicotinic acid, also known as Vitamin B3 (niasin) and pyridine-3-carboxylic acid are the most important ones and are used as antihyperlipidemic drugs and agents for reducing cardiovascular risks [4-5]. It is reported that the copper complexes of the nicotinic-carboxylic acid derivatives mimic the SOD activity [4-5]. The studies made showed that the bioactive pyridine derivatives containing appropriate N- and O- electron donors and hydroxypyridines, aminopyridines and picolinic acid (pyridine-2-carboxylic acid) and their compounds have important antiviral [6], antifungal [7] and antibacterial [8] activity as they induce apoptosis [9] and immune system response [10]. Such properties may play important role in developing new antimicrobial drugs [11, 12]. Another pyridine derivative – 2-amino-3-cyanopyridine – was found to have antibacterial [13], antimicrobial [14, 15], antifungal [16], cardiotonic [17], analgesic [18], and anti-inflammatory [19] properties and was effective against lung cancer [20]. The studies showed that induction of apoptosis was related with the antimicrobial mechanism and the antibacterial effect occurred through apoptosis [21]. Still so little is known even about the antibacterial properties of *cis*-dichlorodiaminoplatinum(II) (cisplatin): one of the strongest anticancer drugs [22]. However, a study showed that this compound has an important antibacterial effect [11]. Taking this into consideration, dichlorodipyridinepalladium(II) ($PdCl_2L^1_2$), dichlorodipyridinenickel(II) ($NiCl_2L^1_2$), dichlorodipyridinecopper(II) ($CuCl_2L^1_2$), dibromodipyridinecopper(II) ($CuBr_2L^1_2$) and dichlorobis-(2,4-dimethylpyridine) copper(II) ($CuCl_2L_2^2$) complexes were shown to have cytotoxic and apoptotic effects [23]. At the same time, they were foreseen to have antimicrobial/antibacterial properties and in a study made by Islam *et al*. [24] the $Ni(C_5H_5N)_2Cl_2$ complex was shown to have considerable antibacterial effect.

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Electrospinning has been reported to be the most efficient and eco-friendly production technique proposed for producing polymer fibers because it provides high surface area-to-volume ratio and possibility of incorporating antimicrobial functionalities into polymers both before and after the process [25, 26] so that to target the microorganisms in question most appropriate fibers could be obtained.

Our group has reported synthesis, characterization and antiproliferative effects of dichlorodipyridine-nickel(II) $[NiCl_2L^1_2]$, dichlorodipyridinecopper(II) $[CuCl_2L^1_2]$, dichlorodipyridinepalladium(II), $[PdL_2Cl_2]$, dibromodipyridinecopper(II) $[CuBr_2L^1_2]$ and dichlorobis-(2,4-dimethylpyridine)copper(II) $[CuCl_2L^2_2]$ were investigated on hepatocellular carcinoma cells [23]. In another study our group synthesized dichlorobispyridinecopper(II) complex – $[CuPy_2Cl_2]$ – and made its characterization studies [27].

Our literature survey showed that this is the first time, CA fibers, which are biocompatible and biodegradable polymers, modified with these complexes are obtained *via* electrospinning and are used against nosocomial infection causing pathogens such as *E.coli, Klebsiellapneumoniae, Methyciline Resistant S. Aureus (MRSA), Enterococcus faecalis.*

2. Experimental

2.1. Synthesis of Complexes

Synthesis of pyridine (L^1) and 2,4-dimethylpyridine (L^2) halide (Cl, Br) metal (Ni(II), Cu(II), Pd(II)) complexes were made as our group had previously published elsewhere [16, 17]. 0.5 mol of MX_2 ($NiCl_2$/$CuCl_2$/$CuBr_2$/$PdCl_2$) salts were dissolved in 40 ml C_2H_5OH under constant stirring while heating the mixture to 343 K. After dissolving 0.25 mol of pyridine/2,4-di-methylpyridine in 20 ml of ethyl alcohol, the solution was added to solutions of the metals studied. After stirring the obtained mixture at constant temperature of 343 K under reflux for 2 h, the solution was cooled to 298 K. Then the complexes were filtered through filter paper and following this they were washed with C_2H_5OH.

Bis(pyridine)dichloronickel(II), $[NiL^1_2Cl_2]$, FT-IR (KBr, ν_{max}, cm^{-1}): 3391–3005 (aromatic C–H stretching vibration), 1647–1364 (Ar–C=C and C=N stretching vibrations), 1241 (C–H in plane bending), 874 (C–H out of bending), 757–634 (C–C in plane bending).^{1}H NMR (D-DMSO, ppm): 8.75 (2H, Ar–C–H), 8.20 (4H, Ar–C–H), 7.20 (4H, Ar–C–H).

Bis(pyridine)dichloropalladium(II), $[PdL^1_2Cl_2]$, FT-IR (KBr, ν_{max}, cm^{-1}): 3405–3007 (Aromatic C–H stretching vibration), 1635–1352 (Ar–C=C and C=N stretching vibrations), 1228 (C–H in plane bending), 865 (C–H out of bending), 745615 (C–C in plane bending). ^{1}H NMR (D-DMSO, ppm): 8.90 (2H, Ar–C–H), 7.90 (4H, Ar–C–H), 6.75 (4H, Ar–C–H).

Bis(pyridine)dichlorocopper(II), $[CuL^1_2Cl_2]$, FT-IR (KBr, ν_{max}, cm^{-1}): 3435–3006 (Aromatic C–H stretching vibration), 1645–1366 (Ar–C=C and C=N stretching vibrations), 1241 (C–H in plane bending), 872 (C–H out of bending), 760–644 (C–C in plane bending).

Bis(pyridine)dibromocopper(II), $[CuL^1_2Br_2]$, FT-IR (KBr, ν_{max}, cm^{-1}): 3455–3004 (Aromatic C–H stretching vibration), 1629–1391 (Ar–C=C and C=N stretching vibrations), 1238 (C–H in plane bending), 867 (C–H out of bending), 756–644 (C–C in plane bending).

Bis(2,4-dimethylpyridine)dichlorocopper(II), $[CuL^2_2Cl_2]$, FT-IR (KBr, ν_{max}, cm^{-1}): 3474–3066 (Aromatic C–H stretching vibration), 2923, 2855 (Methyl–C–H), 1623–1451 (Ar–C=C and C=N stretching vibrations), 1276 (C–H in plane bending), 821 (C–H out of bending), 724-572 (C–C in plane bending).

2.2. Preparing CA Gel

CA gel was obtained by dissolving 6 g of CA (MA 30000 g/mol) and 0.1 g of pyridine-halide metal complexes in 50of ml acetone by stirring for 1 h at 298 K.

2.3. Electrospinning of CA/$[ML_2X_2]$ Microfibers

The CA/$[ML_2X_2]$ microfibers were obtained by electrospinning system at room temperature. The CA/$[ML_2X_2]$ microfibers were obtained from 6 g of CA containing different amounts of pyridine derived metal halogen complexes under an applied voltage of 12 kV at the flow rate of 0.51 ml/min and the distance between the collector and the nozzle was 10 cm.

2.4. Investigation of the Antibacterial Activities of the Complexes and the Microfibers Bacteria Strains

Extended Spectrum Beta Lactamase (ESBL), *K. Pneumoniae, E. Faecalis,* gram negative *E. Coli* (ATCC 35218) and gram positive *MRSA* (clinical isolate), which are the bacteria strains known to be the cause of nosocomial infections, have been used for studying the antibacterial property of the pyridine-halide metal complexes and the CA-metal complex fibers. After incubating the bacteria in nutrient broth at 310 K for 24 h, before investigating the antibacterial property, the said bacteria were inoculated on nutrient agar plates in an incubator at 310 K for 24 h.

2.5. Antibacterial Property Study

Antibacterial properties of the complexes were studied against the bacteria strains *via* disc diffusion and broth microdilution methods (CLSI-2012a, CLSI-2012b) [28, 29]. Besides this, Antibacterial Activity Test of Fabrics Method (JIS L 1902:2002) was used to study the antibacterial property of the CA fibers containing these complexes [30]. In the Disc Diffusion Method, the Mueller-Hinton agar surfaces were covered with bacterial suspensions, the turbidity of which was adjusted to 0.5 McFarland (~10^8 cfu/ml) in Mueller-Hinton Broth. The 25 mg/ml solutions of the complexes, which were prepared by dissolving them in DMSO, were filtered and sterilized. These solutions were then absorbed on empty discs so that there would be 1 mg of complex on each disc. Following this, the discs containing 40 µl of complexes were dried at 303 K in an oven. Then, upon taking them *via* sterile forceps they were placed on agar surfaces. As reference antibiotic Gentamicin discs (10 µg/disc, Bioanalyse) were used and as negative control 5% DMSO was used. After incubating the media at 310 K for 24 h the inhibition zones with diameter > 7 mm were recorded. Experiments were performed in three replicates and the mean and standard deviation were calculated.

Broth Microdilution Method was performed on a 96-well microplate system by adding 100 µl of 2.5 mg/ml cation solutions in DMSO adjusted Mueller-Hinton Broth (BBL) to each well. Then, before they were added to the wells in the first row they were filtered and sterilized. Each experiment was made in triplicates. 10 µl of the bacterial suspensions with the concentration of $5.0 \cdot 10^6$ cfu/ml was added to each well after making two-fold serial dilutions to provide a concentration in the range between 1.25–0.195 mg/ml. Besides the growth control group a well without complexes and sterility control – a well without bacteria and complexes – was used for each bacteria strain. As reference antibiotic Gentamicin (40 µg/ml) (Sigma) was used. The MIC value to be used for each complex was determined by determining the concentration of the well that was not turbid after incubating the microplates at 310 K for 20–24 h.

In the Antibacterial Activity Test of Fabrics Method, after sterilizing 0.4 g of the microfibers with UV light, the sterile CA microfibers – negative control – and the CA microfibers modified with the complexes were placed in test tubes. Then the test tubes, into which 200 µl of suspensions of bacteria adjusted to ~$1.5 \cdot 10^5$ cfu/ml were added, were incubated at 310 K for 18 h. Then, into each test tube 10 ml of the sterile neutralization solution containing 0.9 % NaCl and 0.2 % Tween 20 was added, and the test tubes were shaken vigorously. Then, from each of the tubes 1 ml of the solution was taken to be placed in nutrient broth containing tubes. Following this, after making 3 serial dilutions, 100 µl solutions taken from each tube was seeded into nutrient agar and incubated at 310 K for 24 h. Each experiment was made in triplicates. After incubation, the average number of bacteria left in the medium containing the bacteria strain and the fiber functionalized with the complexes was determined. The antibacterial property of the modified fibers was determined *via* the below given formula:

$$\text{Reduction of bacteria (Log cfu)} = \text{log cfu (negative control)} - \text{log cfu (microfiber containing complex)}$$

$$\% \text{ efficacy} = (\text{cfu/ml at 0 h} - \text{cfu/ml at 24 h}) / (\text{cfu/ml at 0 h}) \cdot 100 \text{ [19]}$$

Antibacterial activity with values less than 0.5, in the range of 0.5–1.0 and >1.0 in the logarithmic decrease was said to not exist, to be slight and to be high, respectively [31].

2.6. Mechanical Properties

A material testing machine (Tinius Olsen H10KS) was used to determine the mechanical properties of the CA/metal pyridine complex containing samples. Shear strength test was made according to ASTM Standard (D3039). The test samples were placed in an air-conditioned room at 296 K with 65% relative humidity over night prior to performing the test.

2.7. Conductivity and Viscosity Measurements

The appropriate fibers were prepared to measure their conductivity (Mettler Toledo, S30k Kit) and viscosity (Viscosimetry, Brookfield, Rvdv-11+Px).

2.8. Statistical Analysis

One-way ANOVA was used for statistical analysis of the results obtained with the disc diffusion method. Differences in results at the $p < 0.05$ were accepted to be significant. Minitab 16 Statistical Software package (Minitab Inc. State College, PA) was used for performing statistical analysis.

3. Results and Discussion

3.1. Structural Characterization Studies

In the FT-IR spectra of the complexes obtained in the range of 4000–400 cm^{-1}, the bands in the range of 3004–3474 cm^{-1} may be due to the stretching vibrations of the C–H in the pyridine ring. Stretching vibrations of the C=C and C–N in the pyridine ring were observed in two different ranges, which were 1623–1647 cm^{-1} and 1352–

1451 cm^{-1}, respectively. The bands observed at 2923, 2870 and 2773 cm^{-1} may be due to CH stretching vibration of $–CH_3$ groups in 2,4-dimethylpyridine. The strong characteristic band observed at 814 cm^{-1} was ascribed to the ring breathing in 2,4-dimethylpyridine. The peak observed in the range from 1240 to 1290 cm^{-1} was due to the in-plane vibrations of δ(C–H) bending vibrations. The bands at 1150 and 1032 cm^{-1} in FT-IR are assigned to C–H out-of-plane bending vibrations. Whereas, the in-plane vibrations of δ(C–C) were observed in two different ranges: 687–754 cm^{-1} and 538–634 cm^{-1}. The peak observed in the range of 754–760 cm^{-1} was attributed to carbon out-of-plane bending vibrations. The selected IR bands are given in Table 1.

Table 1

The selected important IR bands of the complexes

Compound	υ (Ar–C–H), cm^{-1}	υ(Ar–C–H), cm^{-1}	υ (Ar–C=C/C=N), cm^{-1}	δ(C–H) in-plane bending	δ(C–H) out-of-plane bending	δ(C–C) in-plane bending
$[Ni(L^1)_2Cl_2]$	3391–3005	–	1647–1364	1241	874	757–634
$[Pd(L^1)_2Cl_2]$	3405–3007	–	1635–1352	1228	865	745–615
$[Cu(L^1)_2Cl_2]$	3435–3006	–	1645–1366	1241	872	760–644
$[Cu(L^1)_2Br_2]$	3455–3004	–	1629–1391	1238	867	756–644
$[Cu(L^2)_2Cl_2]$	3474–3066	2923, 2855	1623–1451	1276	821	724–572

In the FT-IR spectrum of the CA microfibers, the bands at 3476, 1736 and 2924–2872 cm^{-1} may be due to –OH, –C=O and $–CH_3$ groups, respectively. In the FT-IR spectra of the complexes, the bands υ(C=C) (1623–1647 cm^{-1}) and υ(C=N) (1352–1451 cm^{-1}) were found to overlap with the vibrations of CA microfibers.

In the ^1H NMR spectra of the complexes aside from the paramagnetic Cu (II) complexes obtained in D-DMSO multiple peaks were observed in the range 6.00–8.00 ppm. They were assigned to pyridine ring (Ar–H, 10H).

3.2. Conductivity and Viscosity

As the concentration of the solution increases, the layer thickness also increases, since concentration is also a factor of viscosity. Only at the right concentration and viscosity continuous fibers can be obtained because only then the entanglement of the polymer fibers and the strong interactions among them offer the favorable process conditions [33]. Surface tension has a pronounced effect on the electrospinning process. For instance, when the viscosity is <<100 mPa·s the number of thin fibers are limited and droplets or beads start to form. However, viscosities >100 mPa·s has a detrimental effect on the electrospinning process and the polymer chains overlap, which in turn hinders fiber formation. In this study the optimum concentration for obtaining fibers were 12 % for CA. The conductivity value of unmodified CA fibers was found to be 6.27 μS/cm. The viscosity and conductivity values of polymer fibers are given in Table 2.

Table 2

Viscosity and conductivity values of the polymer fibers

Polymer solution	Viscosity, mPa·s	Conductivity, μS/cm
CA	8.77	6.27
CA-$[Cu(L^1)_2Cl_2]$	7.95	34.9
CA-$[Cu(L^1)_2Br_2]$	8.02	33.6
CA-$[Ni(L^1)_2Cl_2]$	7.99	35.5
CA-$[Pd(L^1)_2Cl_2]$	8.50	34.3
CA-$[Cu(L^2)_2Cl_2]$	7.30	37.2

3.3. Imaging Studies

The microscope images of CA microfibers modified with the $[ML_2X_2]$ complexes were prepared and their microscope images at 16× were taken and are presented in Fig. 1. The microscope images revealed that the average diameter of the fibers lied in the range of 6–25 μm.

The scanning emission microscope (SEM) images of CA microfibers modified with metal complexes are given in Fig. 2. The thicknesses of the microfibers lie in the range from 0.2 to 10 μm.

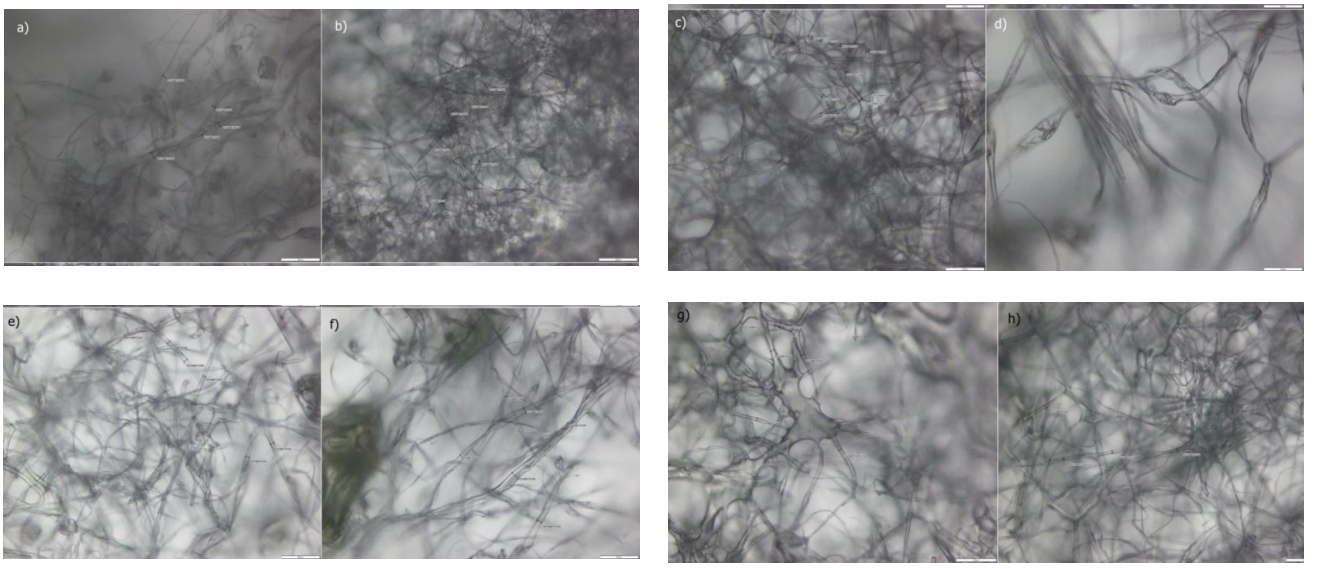

Fig. 1. The microscope images of microfibers containing complexes at different amounts: 0.04 g [NiL_2Cl_2] (a); 0.08 g [NiL_2Cl_2] (b); 0.05 g [PdL_2Cl_2] (c); 0.1 g [PdL_2Cl_2] (d); 0.05 g [CuL_2Br_2] (e); 0.1 g [CuL_2Br_2] (e); 0.05 g [CuL_2Cl_2] (g) and 0.1 g [CuL_2Cl_2] (h)

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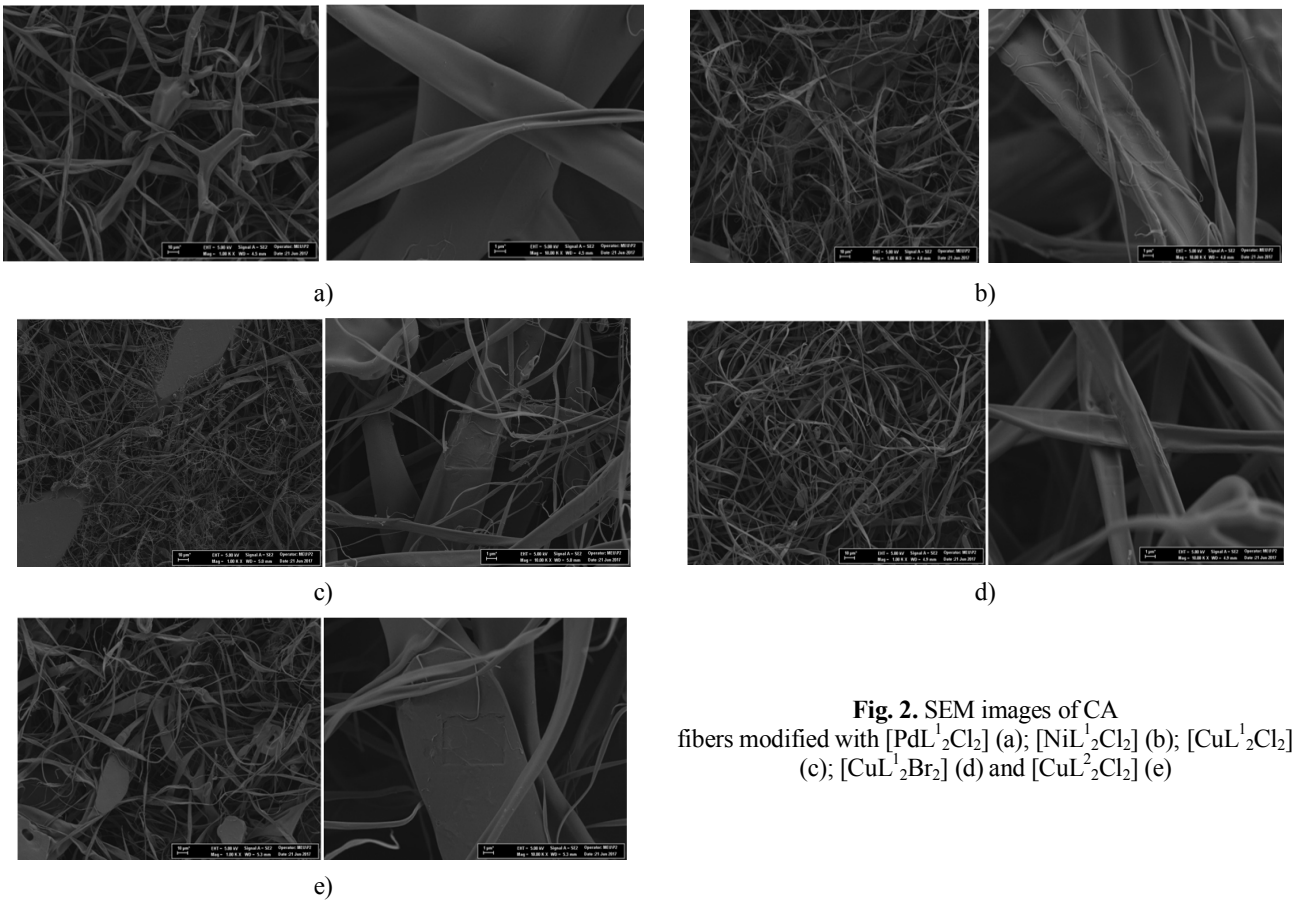

Fig. 2. SEM images of CA fibers modified with [$PdL^1{}_2Cl_2$] (a); [$NiL^1{}_2Cl_2$] (b); [$CuL^1{}_2Cl_2$] (c); [$CuL^1{}_2Br_2$] (d) and [$CuL^2{}_2Cl_2$] (e)

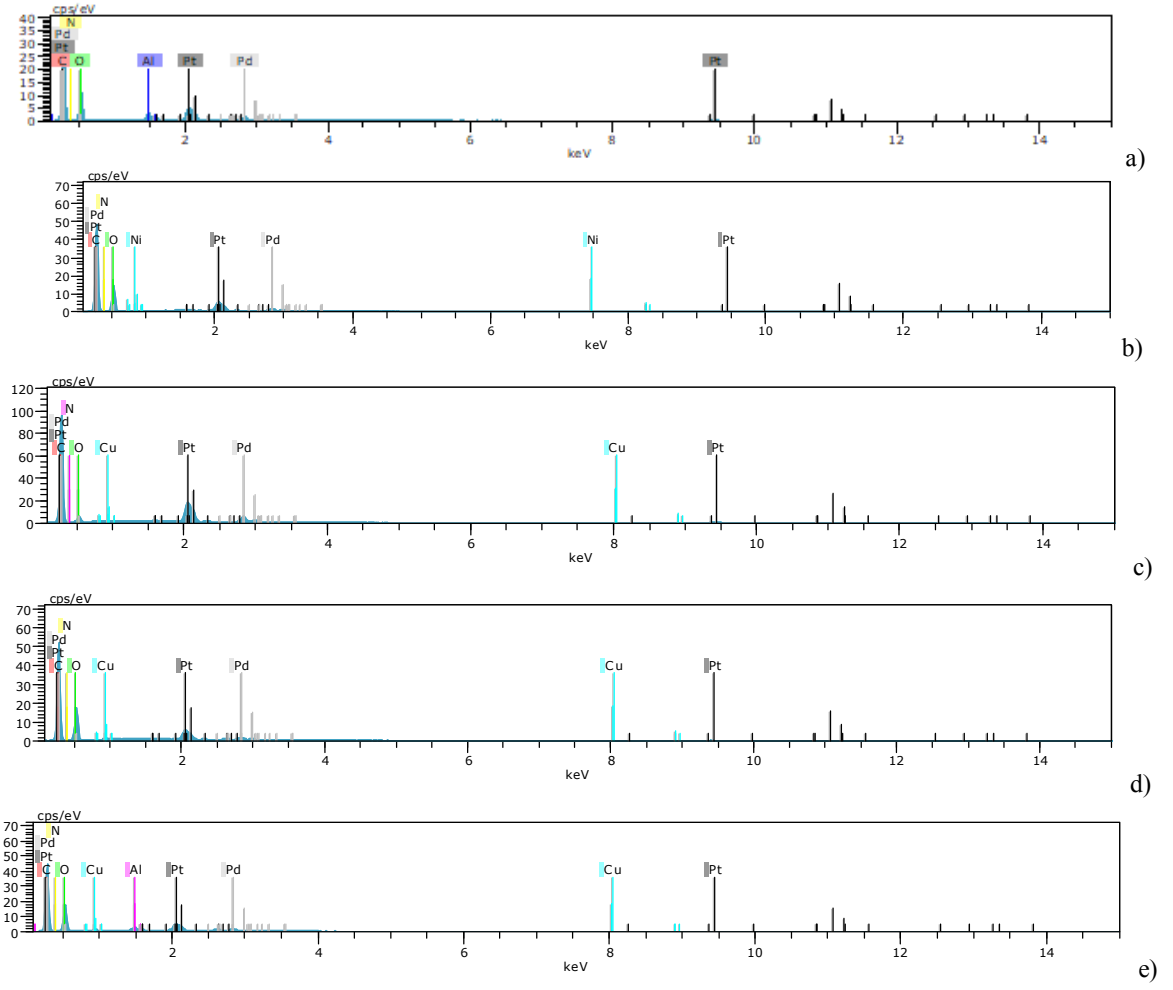

Fig. 3. EDX spectra of the CA fibers modified with $[PdL^1{}_2Cl_2]$ (a); $[NiL^1{}_2Cl_2]$ (b); $[CuL^1{}_2Cl_2]$ (c); $[CuL^1{}_2Br_2]$ (d) and $[CuL^2{}_2Cl_2]$ (e)

The EDX spectra of the CA fibers modified with the complexes are given in Fig. 3. The microfibers were covered with platinum for SEM-EDX studies. Therefore, platinum and palladium peaks were also observed in the spectra. The results indicate that the fibers were successfully modified with the complexes.

3.4. Mechanical Behavior of Microfibers

Since the microfibers were thin and were not robust, among the methods which are applied on the textile materials such as fabrics only shear strength test (D3039) could be applied, and the results obtained are given in Table 3. Prior to analysis the test samples were kept in an air-conditioned room at 296 K with 65% humidity over night. The shear strengths of the CA microfibers were found to be 34.40 N. It was observed that the shear strengths of the microfibers modified with the complexes was observed to be higher than that of the unmodified ones. The hydrogen interactions among the functional groups in the CA monomers (–CO and –OH groups), and those in the complexes ($–NH_2$ and $–NO_2$) have strengthened the bonds in the polymers. This in turn resulted in increase in shear strength. The shear strength of the CA/complex fibers as given in Table 3 was found to be in the range of 53.30–67.50 N.

Table 3

Shear strength of the microfibers

Compound	Shear strength, N
CA	34.40
$[CuL^1{}_2Cl_2]$-CA	65.10
$[CuL^2{}_2Cl_2]$-CA	53.30
$[CuL^1{}_2Br_2]$-CA	57.30
$[PdL^1{}_2Cl_2]$-CA	66.90
$[NiL^1{}_2Cl_2]$-CA	67.50

3.5. XRD Studies

The XRD patterns of CA microfibers in various solvent (acetone, dichloromethane and dimethylsulfoxide) are given in Fig. 4. The XRD patterns of CA fibers revealed that the fibers had amorphous structure. The broad peaks observed at 2θ values corresponding to 19–23° indicate that the polymer solution is also crystalline in nature. While more crystalline structure is formed when acetone is used as solvent it was observed that crystallinity in dichloromethane was very little.

3.6. Antibacterial Studies

The reproduction inhibition zone diameters obtained in the disk diffusion test made for investigating antibacterial activity of the complexes and the results of one-way variance analysis (ANOVA) are given in Table 4. When the activities of the complexes against the bacteria were compared the differences among the groups were found meaningful at level $p < 0.05$. It was observed that $[NiL^1{}_2Cl_2]$ and $[CuL^1{}_2Br_2]$, which are in the same group, were found to be effective against *E. Coli*. $[CuL^1{}_2Cl_2]$, $[CuL^1{}_2Br_2]$, $[CuL^2{}_2Cl_2]$ and $[NiL^1{}_2Cl_2]$ were found to be effective against *S. Aureus* and *E. Faecalis*, respectively. $[PdL^1{}_2Cl_2]$ was found to be effective against *E. Coli, K. Pneumoniae* and *S. Aureus*.

In order to determine the minimum inhibitor concentration of the complexes effective on the bacteria and to verify the disc diffusion test results, microdilution test was made. The MIC results are presented in Table 5. According to the results obtained, all active agents were found to have different MIC levels in the range of 6.25–0.78 mg/ml. The lowest MIC values, obtained for 5 active agents tested against four different bacteria, were as following: $[PdL^1{}_2Cl_2]$ against *E.coli*, $[NiL^1{}_2Cl_2]$, $[CuL^1{}_2Cl_2]$ and $[CuL^2{}_2Cl_2]$ against *K. Pneumoniae,* $[CuL^1{}_2Cl_2]$, $[CuL^1{}_2Br_2]$ and $[CuL^2{}_2Cl_2]$ against *S. Aureus* and $[Ni(L^1)_2Cl_2]$, $[Cu(L^1)_2Cl_2]$ and $[Cu(L^2)_2Cl_2]$ against *E. Faecalis.*

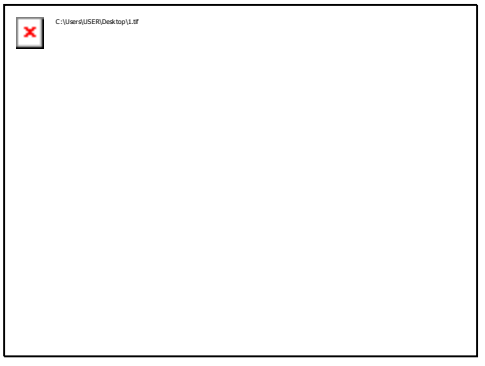

Fig. 4. XRD pattern of CA polymer

Table 4

Reproduction inhibition zone diameters (mm) of the complexes were found according to Disc Diffusion Method

Complexes (4mg/disc)	Antibacterial activity zone diameter, mm			
	**E. Coli*	**K. Pneumoniae*	***S. Aureus*	*E. Faecalis*
$[NiL^1{}_2Cl_2]$	18 ± 1^{Ab}	19 ± 0.57^{Aab}	12 ± 0.57^{Cc}	20 ± 0.57^{Aa}
$[CuL^1{}_2Cl_2]$	16 ± 0.57^{Bb}	16 ± 1.52^{ABb}	20 ± 1.52^{Ba}	18 ± 1.52^{Bab}
$[CuL^1{}_2Br_2]$	18 ± 0.57^{Aa}	14 ± 2^{Bb}	19 ± 0.00^{Ba}	13 ± 1^{Cb}
$[PdL^1{}_2Cl_2]$	14 ± 0.57^{Ba}	15 ± 0.57^{Ba}	14 ± 0.57^{Ca}	11 ± 0.57^{Cb}
$[CuL^2{}_2Cl_2]$	15 ± 0.00^{Bc}	17 ± 0.57^{ABb}	23 ± 0.57^{Aa}	18 ± 0.57^{Bb}
Gentamisin (30µg /disc)	24±0.6	22±0.6	19±0.0	23±0.6

Notes: *broadened spectrum beta lactamase; **Methyciline resistant.
Average±standard deviation A-C: differences between the groups in the same column;
a-c: differences between the groups in the same line, meaningful at $p < 0.05$ level.

Table 5

Minimum inhibitor concentration (MIC) values determined *via* broth microdilution method for the active agents

Complexes (50 mg/ml)	Minimum inhibitor concentration (MIC), mg/ml			
	**E. Coli*	**K. Pneumoniae*	***S. Aureus*	*E. Faecalis*
$[NiL^1{}_2Cl_2]$	3.12	1.56	3.12	1.56
$[CuL^1{}_2Cl_2]$	3.12	1.56	1.56	1.56
$[CuL^1{}_2Br_2]$	3.12	3.12	1.56	3.12
$[PdL^1{}_2Cl_2]$	0.78	3.12	3.12	3.12
$[CuL^2{}_2Cl_2]$	1.56	1.56	1.56	1.56
Gentamisin (30µg /disc)	0.00312	0.00312	0.00625	0.00625

Notes: *broadened spectrum beta lactamase; **Methyciline resistant

Table 6

According to JIS L 1902 method the number of colony forming bacteria (CFB) for the CA fibers

Test Material CA	CFB after incubation			
	**E. Coli*	**K. Pneumoniae*	***S. Aureus*	*E. Faecalis*
Reproduction control	$3.0 \cdot 10^6$	$1.3 \cdot 10^6$	$1.0 \cdot 10^6$	$8.4 \cdot 10^5$
Antibacterial textile	$2.1 \cdot 10^2$	$3.2 \cdot 10^2$	$1.1 \cdot 10^2$	$2.5 \cdot 10^2$
$[Ni(L^1)_2Cl_2]$/CA	$5.3 \cdot 10^3$	$9.1 \cdot 10^3$	$35 \cdot 10^3$	$4.5 \cdot 10^3$
$[Cu(L^1)_2Cl_2]$/CA	$7.4 \cdot 10^3$	$15 \cdot 10^3$	$3.0 \cdot 10^3$	$8.0 \cdot 10^3$
$[Cu(L^1)_2Br_2]$/CA	$8.2 \cdot 10^3$	$20 \cdot 10^3$	$4.5 \cdot 10^3$	$50 \cdot 10^3$
$[Pd(L^1)_2Cl_2]$/CA	$6.1 \cdot 10^3$	$30 \cdot 10^3$	$15 \cdot 10^3$	$85 \cdot 10^3$
$[Cu(L^2)_2Cl_2]$/CA	$6.1 \cdot 10^3$	$12 \cdot 10^3$	$2.1 \cdot 10^3$	$11 \cdot 10^3$

Notes: *broadened spectrum beta lactamase; **Methyciline resistant, cfb: Colony forming bacteria number

Table 6

Antibacterial activity results obtained according to JIS L 1902 method for the fibers

Test Material CA	Reproduction decrease log cfb			
	**E. Coli*	**K. Pneumoniae*	***S. Aureus*	*E. Faecalis*
$[NiL^1{}_2Cl_2]$/CA	2.778	2.046	1.456	2.250
$[CuL^1{}_2Cl_2]$/CA	2.632	1.824	2.523	2.000
$[CuL^1{}_2Br_2]$/CA	2.574	1.699	2.347	1.204
$[PdL^1{}_2Cl_2]$/CA	2.699	1.523	1.824	0.974
$[CuL^2{}_2Cl_2]$/CA	2.699	1.921	2.699	1.862

Notes: *broadened spectrum beta lactamase; **Methyciline resistant (<0.5 means no antibacterial activity, 0.5–1.0 means low antibacterial activity and >1.0 means high antibacterial activity)

To determine the antibacterial property of the microfibers containing the active agents, the test method used for determining the antibacterial activity of textiles (JIS L 1902:2002) was exploited. According to the results obtained, the average number of colony forming bacteria at the end of 24 hours is given in Table 6.

The antibacterial activity results for the textile fibers are given in Table 7**.** According to these results, it is seen that all the fibers showed antibacterial effect.

4. Conclusions

In this research $[ML_2X_2]$ complexes were prepared, and their structures were characterized via FT-IR, ^{1}H NMR and ^{13}C NMR techniques. CA fibers modified with complexes by adding them at their respective MIC amounts were prepared via electrospinning. FE-SEM images of the microfibers containing complexes revealed that the microfibers had an average diameter of 0.05–10 micrometers, but they were not homogeneous. Modification of the CA microfibers modified with the complexes was revealed via EDX analysis. The EDX results, which showed that the microfibers contained the elements Ni, Cu, Co, C, O, and N, revealed that the complexes were distributed in the microfibers. The mechanical properties of the fibers were determined according to D3039 ASTM test method – the Standard Test Method for Tensile Properties of Polymer Matrix Composite Materials. The tensile strength of the unmodified CA microfibers and CA fibers modified with the complexes were found to be 34.40 and 53.30–67.50 N, respectively. The physical characterization of the microfibers was made by FE-SEM analysis.

Anti-bacterial studies were performed against the bacteria *E. Coli, Klebsiella Pneumoniae, Methyciline Resistant Staphylocacous Aureus (MRSA), Enterococcus Faecalis via* modified disc-diffusion method (CLSI- M02-A11). The MIC of the modified microfibers was determined via broth microdilution method (CLSI-M07-A9) to determine the amount of the pyridine derivatives that should be added to the CA solution. Antibacterial activity of both the complexes and the microfibers were determined via disc agar diffusion method JIS L 1902:2002, testing method for antibacterial activity of textiles. The microfibers, which were taken to contain equal amount of active agent per unit weight, were placed on agar plates. The results indicated that all the complexes and the CA fibers containing these complexes showed *in-vitro* antibacterial activity against the nosocomial vectors. Statistically high antibacterial activity was observed with $[Ni(L^1)_2Cl_2]$ and $[Cu(L^1)_2Br_2]$ complexes against *E.coli*and, $[Ni(L^1)_2Cl_2]$ against *K. Pneumoniae,* $[Cu(L^2)_2Cl_2]$ against *S. Aureus*, and $[Ni(L^1)_2Cl_2]$ against *E. Faecalis.* The results showed that the CA microfibers, when modified with these

complexes at their MIC values, had high bacteriostatic and bactericidal properties and therefore have great potential to be used for producing antibacterial textiles with high effectiveness against MRSA.

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АНТИБАКТЕРІАЛЬНІ АЦЕТАТЦЕЛЮЛОЗНІ МІКРОВОЛОКНА, ЩО МІСТЯТЬ ПОХІДНІ КОМПЛЕКСИ ПІРИДИНУ

Анотація. *Синтезовано галідні комплекси піридину (L^1) та 2,4-диметилпіридину (L^2) типу $[ML_2X_2]$. За допомогою Фур'є-спектроскопії та ^{1}H ЯМР визначено їх характеристику. Ацетатцелюлозні волокна, що містять синтезовані комплекси, були електроспіновані та досліджені з використанням Фур'є-спектроскопії, а їх морфологію визначено за допомогою FE-SEM. Досліджено антибактеріальну активність комплексів та волокон.*

Ключові слова: *електроспінінг, мікроволокна, антибактеріальний, піридинові комплекси, ацетат целюлози.*