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Antibacterial Activity and Conductivity Properties of Nanocomposites based on Cellulose Acetate Nanofibers and Copper Nanoparticles

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Abstract

In this work, nanocomposites comprising copper nanoparticle in cellulose acetate (CA) matrices have been prepared. In this manner, Copper nanoparticles prepared by its salt reduced by sodium borohydride at various concentration. Then this nanoparticle solution was mixed with polymer solution and electrospun by electrospinning device. The above nanocomposite has been successfully detected by SEM, EDAX, FT-IR and UV-Vis Spectroscopy and indicated that not only nanocopper and cellulose nanofiberhave created good chemical interaction but also a relatively suitable size and distribution has been yielded in the fibers. The resulted nanocomposite conductivity by four-probe method showed a remarkable conductivity produced by nanocopper distribution in the tissue of nanocomposites increasing as concentration rises. In addition, it has been realized that the above nanocomposites have good antibacterial properties against Staphylococcus aureus (gram-positive) and also Escherichia Coli and Klebsiellapneumonia (gram-negative) bacteria.

***Keywords**: Cellulose acetate, Nanocomposite, Copper nanoparticles, Conductive properties, Electrospinning.*

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Introduction

Studies on the minerals /organic nanocomposites compounds are growing and advancing in the interdisciplinary field of materials science and engineering and particularly, the research on developing sustainable and environmentally friendly resources has got widespread. The main idea in this direction is to produce nanocomposites consisting of biopolymers that can be substituted with synthetic polymers in special fields.

Consequently, the polymers including starch, cellulose, dextran and chitosan due to being biodegradable and renewable in nature and because of the presence of various functional groups are used [1,3]Through the relationship cellulose has with diverse fillers, we can access such merits as the boosted properties (optical, mechanical, and electrical conductivity and etc.) and some unique applications [4,5]. Among a broad spectrum of the existing mineral fillers, metal nanoparticles (gold, silver, and copper among others) possess unique properties [6]. Compared with bulk similarity in terms of their different size and surface effects, metal nanoparticles show some unique properties .As a result, the final properties of composite materials can be considered as a function of size, shape, particles size distribution and also the interaction with cellulose matrix[7].

One of the methods to produce nanoparticles is the reduction of metal salts [8-10]. The most important method for producing nanofibers is electrospinning due to its high variety and the convenience using the device [11]. Many polymers have been transformed into nanofibers via this method [12]. since the availability of nanoparticles resulted in the improvement of the nanofibers' properties and composite nanofibers' applicability [13,14].On the other hand, most of the polymers are insulate electrically speaking; however, the conductive metal nanoparticles supply a satisfactory conductivity in the nanocomposites of metal & polymer [15].

It is viable to apply the conductive nanocomposites in diverse applications including rechargeable battery, shield, sensor, electrode and etc. [16, 17].Al so, the bacterial adhesion and cell culture studies showed that the polymer/metal nanocompositescontrol the antibacterial activity and improve the biocompatibility compared to the virgin polymers [18].

According to the conducted studies, so far cellulose acetate nanofibers with copper nanoparticles (produced by reduction) haven’t been produced by electrospinning and also no work has been done on directing such nanocomposites. In the current research, composite nanofibers containing copper nanoparticles through copper salt reduction and following it, electrospinning of polymer solution has been supplied and its conductivity traits have been

measured. In addition, the previously proved properties of copper nanoparticles as anti-bacterial, anti-viral and anti-fungal [19-20], have undergone anti-bacterial tests, yielding fulfilling results.

Experimental

Material

Copper(I) nitrate and sodium borohydride were purchased from Merck Co. (Germany), cellulose acetate (CA) (39.8 wt% acetyl, average Mn ~30,000) were purchased from Sigma-Aldrich,all solution were used as purchased without further purification.

Devices and Method

Fourier transform infrared spectroscopy (FTIR), Bruker Tensor 27 (Germany) has been used for analyzing the chemical structure and functional groups and the synthesis process influencing the samples' functional groups. To survey the surface of the fibers, Scanning Electron Microscope (SEM), Philips XL 30(Germany) has been employed and gold coating with a coating time of 60 s has been used in order to prepare the samples. To produce nanofibers, electrospinning device KATO TECH (Japan) has been applied. Energy-dispersive X-ray spectroscopy (EDS), Samx England has been employed to detect the fibers production ingredients. UV-Vis Absorption Spectrophotometer, Cary 100 Bio, England has been used for measuring absorption wavelengths in nanocomposite samples' solutions. The antibacterial properties have been carried out by AATCC 100-1993 method. In this method, bacterial culture solution at concentration 10^5 CFU/ml has been transferred to the tube and then the sample has been incubated. Also its humidity and temperature has been controlled. After 24 h, it has been diluted and 1cc of the solution has been added to the plated containing 25 ml sterile Notrite agar and after homogenization, it has been incubated for 18-24 h at 37ºC. After that the plates have been removed from the incubator and the live cells have been counted. To calculate Bacteria reduction rate, Equation1 has been applied.

$$\%R = \frac{CFU\ Blank\ Nanofiber - CFU\ Nanocomposite}{CFU\ Blank\ Nanofiber} \times 100$$

%R: percent Reduction of colony

Equation1. Computing bacteria drop rate %.

The prepared nanocomposite conductivity ASTM45 USA was used by four-probe method. In this method, nanocomposite is converted into pellets under pressure. The four-probe method has four needles, which get located on this pellet. A constant voltage is applied on the fibers, the current and output voltage is measured and the fibers' conductivity is obtained this way.

Composite Nanofibers Production Method

Copper nanoparticles were synthesized using copper (II) nitrate and sodium borohydride in a molar ratio of 6:1. 250 mg of $Cu(NO_3)_2 \cdot 3H_2O$ was dissolved in 70 ml of CH_3CN and 30 ml of purified water. Under vigorous stirring, freshly prepared sodium borohydride solution was injected drop by drop into the Nitrogen-bubbled reaction medium via a syringe pump. The injection of the sodium borohydride solution was continued (8 ml) until color change was observed in wine-reddish color. After the reduction was completed, the solution was centrifuged and the particles were separated by washing with methanol and dried in a vacuum [21]. Then in beaker and at 60°C, acetate cellulose (to prepare solution 10% W/v) is added to a solution of 25 ml including acetonitrile 50% and DMF 50% and put in shaker for 4h in order to get totally dissolved and homogenized.

Then a certain quantity of copper nanoparticles is added to the above solution to prepare the concentrations as 300, 500, 1000, 1500& 2000, of the above nanoparticles to 50ml of the solution. and the solution is placed in the ultrasonic homogenizer so that in addition to getting homogenized, lead to the probable full bonding of nanoparticles and polymer. After that, the above solution has been converted into nanocomposite nanofiber containing nanoparticles using electrospinning device. This way that the solution mixture has been poured into a 5 ml syringe and voltage 25 kV has been imposed between syringe needle and drum collector. The noted solution feed rate has been set 0.48 ml/h. The syringe tip has been placed 15 cm away from the drum. The rotating spinning has been set at 2 RPM and the ambient temperature of the device has been reported 25 ° C.

Results and discussion

Fourier Transform Infrared Spectroscopy (FT-IR)

In the blank sample (nanofibers of cellulose acetate) demonstrated in the spectra of Figure.1 and 2with letter a, there are two strong peaks in the area 1031 and 1686 cm^{-1},indicating the acetyl group ,the absorption band 3591cm^{-1} belongs to group H-O. Symmetric and asymmetric vibrations of CH_3 are spotted at 1323 cm^{-1} and 1404 cm^{-1}[22-24] (Figure 1 spectrum a).Considering IR spectra of electrospun sample of blank fibers (nanocopper free)

and composite nanofibers 300ppm , the change of index peaks ,in particular those of the carbonyl group is seen. For instance, the peak related to blank sample has been shifted from 1031 and 1689 cm^{-1} to 1043 and 1742 cm^{-1}, respectively and shifted with a CH_3 group peak related to the blank sample acetyl group from 1323 $cm^{-1}$to 1372 cm^{-1} in the sample of 300ppm copper. Besides, the peaks intensity has increased in the samples containing 300ppm nanoparticles (Figure 1).

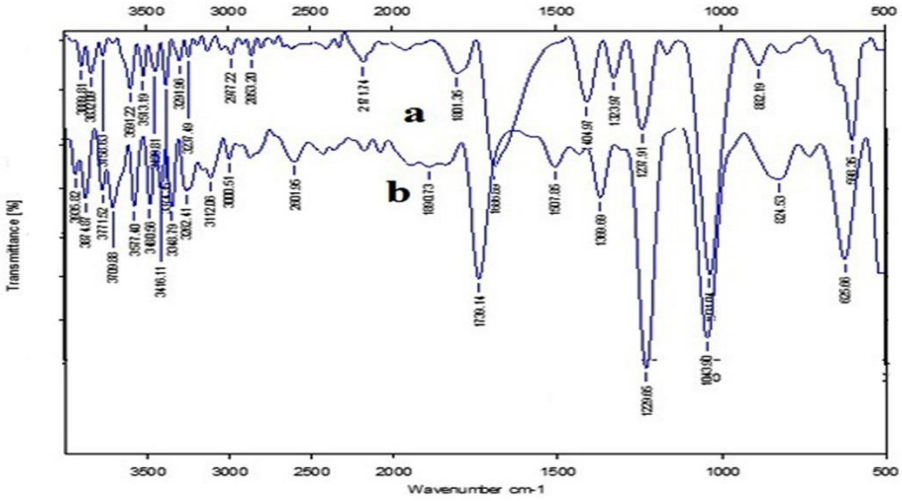


Figure1.IR spectrum of composite nanofibers:(a) blank sample (electruspun nanofiber without nanoparticle); (b) composite nanofibers sample at concentration 2000ppm of copper and silver nanoparticles.

Observing the samples spectra with the higher concentration of nano copper particles, i.e., 500, 1000, 1500 and 2000 ppm relative toblankspectrum, the mentioned index peaks intensity have increased significantly The peaks shifting and their intensity rise imply nano copper penetrating nanofibers and creating strong chemical interaction with it (Figure 2).

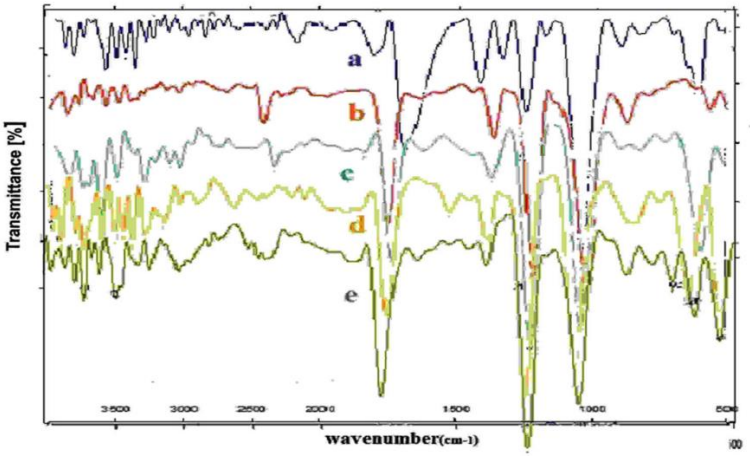


Figure 2. The in situ nanocomposite IR spectra: a) the blank sample & with copper nanoparticles at concentration b) 300 ppm c) 500 ppm c) 1000 ppm d) 1500 ppm e)2000 ppm.

Scanning Electron Microscope (SEM)

In the studies related to nanocomposites material properties, electron microscope is one of the best and the most applied devices used. In fact, using electron microscope and SEM images, it is possible to monitor nanoparticles placement on the composite nanofibers and prove their size and distribution manner. As seen in SEM images, the fibers' diameter in nm. The distribution of the copper nanoparticles is optimally uniform. Moreover, the size of nanoparticles is in nm. Via closer monitoring, it is determined that their size is smaller in lower concentrations of nanoparticles. For example, in the concentration 500 ppm, they are in the size 45-55mm while at concentration 2000 and 1000 ppm, their average size gets bigger reaching around 65-75 mm. This is because when nanoparticles concentration increases, their adhesion goes up (Figure3).

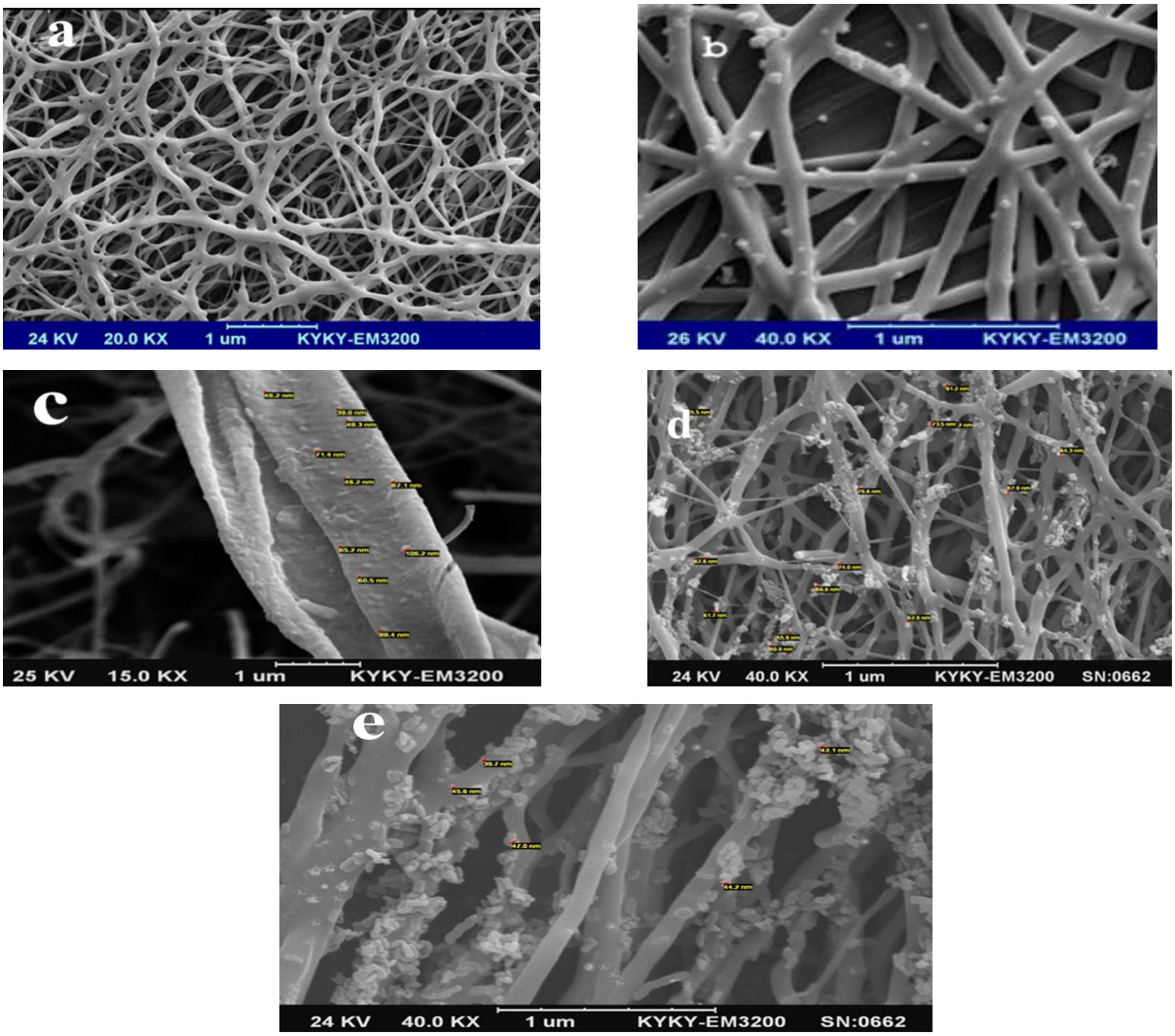


Figure 3. SEM image of nanocomposite sample at acetate cellulose concentration 15% w/v(a) blank sample and at copper nanoparticles concentration (b) 300 ppm (c) 500 ppm (d) 1500 ppm (e) 2000 ppm.

UV -Vis Spectroscopy

The producing and size of Cu/NPs copper nanoparticles as well as their relative distribution can be followed and demonstrated through UV Vis spectrum so that the absorption peaks around 550-600 nm can be attributed to the production of nanoparticles [25]. While there was no peak in this area before adding sodium borohydride to CA/$CuNO_3$,After adding the reducing agent, absorption peaks are observed in this area, The reason supports the formation of nanoparticles observed in this area and besides, their size can be obtained based on the maximum wavelength (λ_{max})[26-28].This way, indicating the maximum wavelength 552 and 555nm for the concentrations 500 and 1500 ppm , copper nanoparticles indicate these particles small size around 45-75 nm (Figure.4). Moreover, given the peaks' sharpness and their width being relatively narrow (about 30-40nm) , the relative distribution of the nanoparticles is uniform[26-28].

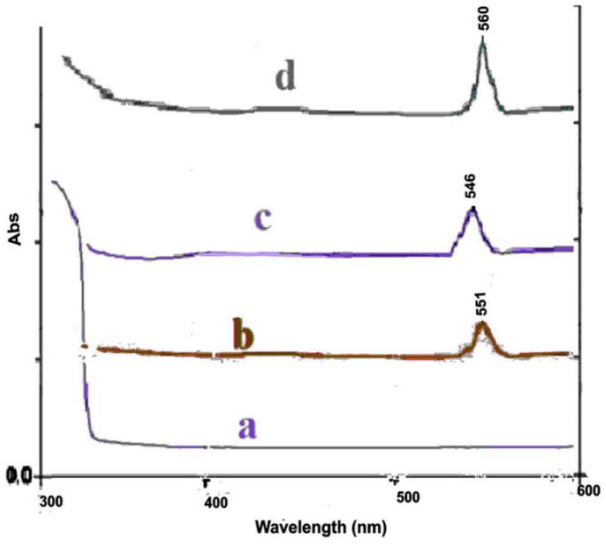
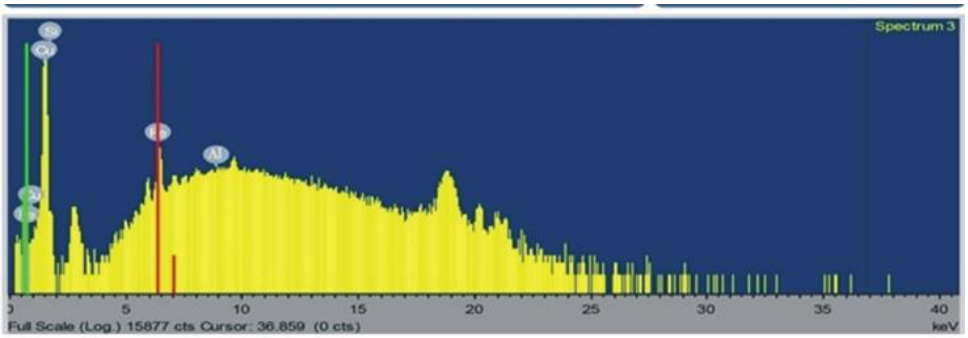


Figure 4. UV-VIS spectrum of composite nanofibers: a) blank sample (electruspunnanofiber without nanoparticle); b) with copper nanoparticles at concentration; b) 500 ppm c) 1000 ppm d) 2000 ppm.

Energy Dispersion of X-ray Spectroscopy (EDS or EDX)

Energy Dispersion of X-ray Spectroscopy (EDX) used to detect the elements existing in a sample has been focused here using this spectroscopy; it is viable to obviously detect the presence of copper in EDS nanocomposite spectrum (Figure 5).



Elem.	Line	Mass[%]	3sigma	Atomic[%]	Intensity[cps/mA]	Formula	Mass[%]	Molecule[%]
29 Cu	K	88.5	0.21	88.62	1382.52	Cu	88.5	88.62
14 Si	K	5.32	0.2	5.25	1.69	Si	5.32	5.25
26 Fe	K	4.34	0.03	4.31	41.38	Fe	4.34	4.31
13 Al	K	1.84	0.02	1.82	4.95	Al	1.84	1.82

Figure 5. EDS spectrum related to composite nanofibers at concentration 1000ppm.

Nanocomposite Conductivity

The nanocomposites` conductivity results have been given in Table 1. As depicted in Table1, the blank nanocomposite (copper nanoparticles free sample) has no conductivity but the specie shaving nanoparticles got conductive. Also the conductivity increases as the copper nanoparticles in the nanocomposite rise.

Table 1. Comparing the synthesized nanocomposites conductivity.

Entry	Nanoparticles concentration in nanocomposites (ppm)	Conductivity of nanocomposite(uS/cm)
1	**0**	**0**
2	**300**	**0.78×10–3**
3	**500**	**6.63×10–3**
4	**1000**	**9.28×10–3**
5	**1500**	**2.26×10–2**
6	**2000**	**5.75×10–2**

Antimicrobial Tests

The antibacterial efficiency of sliver surface only works at high temperature (35ºC) and high humidity (90% or higher relative humidity). While the surface of copper at room temperature (22º C) and normal humidity (50%) is able to kill 99.9% of microbes, thus antibacterial test has been done with nanocomposites, yielding satisfactory results [29-30].

Therefore, the antibacterial properties have been performed with gram-positive bacteria as Staphylococcus aureus, and gram-negative ones such as EscherichiaColi and Klebsiellapneumoniae. The results have been obtained from 5 iterations showing the bacteria growth rate variation percentage. Regarding the antibacterial results, it has been determined that nanocomposite has significant antimicrobial properties and also, as copper`s concentration increases in the nanocomposite, the antibacterial properties get more.

Table 2. the synthesized nanocomposites antibacterial results.

Entry	Nano copper concentrations on nanocomposites (ppm)	Staphylococcus aureus percent reduction of colony (R%)	Escherichia coli percent reduction of colony (R%)	Klebsiellapneumoniae percent reduction of colony (R%)
1	**300**	**67**	**43**	**66**
2	**500**	**79**	**40**	**70**
3	**1000**	**75**	**52**	**61**
4	**1500**	**82**	**55**	**68**
5	**2000**	**92**	**66**	**70**

Results and discussion

The nanocomposites of CA/ Cu NPs have been prepared successfully by electrospinning out of cellulose acetate nanofibers containing various amounts of Cu NPs nanocopper have resulted via the reduction of nitrate copper (I) by Sodium borohydride on the cellulose acetate polymer solution. Various tests have successfully been employed to detect the mentioned nanocomposite.SEM images have showed that copper nanoparticles have uniformly spread, with sizes 45-75nm in the nanocomposite texture. This is verified by UV spectroscopy. The EDS spectrum. FT-IR spectrum indicates strong interaction between nanocopper and nanofibersso that the index peaks also have had significant shifts and the peaks' intensity has changed, too. Moreover, it has been determined that due to the presence of copper in the nanocomposite tissue, high conductivity to cellulose acetate has resulted that as the concentration of copper nanoparticles in nanocomposite increases ,this conductivity goes up, too.And ultimately, due to the presence of nanocopper against the gram-positive bacteria such as Staphylococcus aureus and thegram-negative bacteria such as Escherichia Coli and Klebsiellapneumonia, its antibacterial properties promise an antibacterial and conductive nanocomposite for future applications.

References

[1] S.V. Manorama,P. Basak, S. Singh, Anti-Microbial Polymer Nanocomposites, T.Trindade,D.A.L. da-Silva,*Nanocomposite Particles for BioApplications*, Pan Stanford: Singapura, 249 (2011).

[2] M.N. Belgacem,A. Gandini,1st Edition from,*Monomers, Polymers and Composites from Renewable Resources*, Elsevier,Amesterdam,Netherlands,321-517(2008).

[3] H. Bang, K. Ma, K. Devarayan, C.Y. Kang, B.k Kim, A. Gopiraman, I.S. Kim, J. Mater. Sci., doi: 10.1016/j.jmst.2016.04.015

[4] J. Shen,Z. Song, X. Qian,Y. Ni, Ind.*Eng. Chem. Res*., 50, 661(2011).

[5] Y. Zare, I. Shabani, Mat. Sci. Eng. C., doi: 10.1016/j.msec.2015.11.023

[6] M.J. Woźniak-Budych, K. Langer, B.Peplińska, Ł.Przysiecka, M.Jarek,M. Jarzębski,S. Jurga,*Mater. Chem. Phys*., 179, 242 (2016).

[7] S.Suresha, S.Karthikeyanc,K.Jayamoorthy,*J . Science: Advanced Materials and Devices*, 1, 343(2016)

[8]C.T. Hsu,C.Wu,C.N.Chuang,S. H.Chen, W-Y. Chiu,K. H. Hsieh,*J. Polym. Res*., 22. 200 (2015).

[9]M.Sargordan, M.Hoseinkhani, F.Ebrahimi., *Research Journal of Pharmaceutical, Biological and Chemical Sciences.*, 5, 155(2014)

[10] I.M.Yakutik,G.P. Shevechenko,*Surface. Science*., 566, 414 (2004)

[11]M.N. Akhtar,A.B.Sulong, A.b. Saniah, C.H. Azhari,M.R.Raza.,*Iran.Polym. J*., 24, 1025 (2015).

[12]M. Khamforoush,R. Agha-Moalapour, *Iran.Polym. J*., 25, 875(2016).

[13]O.E. Fayemi, A.S.Adekunle,E.E. Ebenso, *J.Nanomater*.,1,10 (2016).

[14]D. j. da Silva,M.T. Escote,S. A. Cruz,D.F. Simião,A. Zenatti,M. S. Curvello, *Polymer Composite*, DOI: 10.1002/pc.24232(2016).

[15]V.Kumar,S. Kalia,Swart,H.C.*Conducting Polymer Hybrids*, C.Tang, N. Nanxi Chen, X. Hu, Conducting Polymer Nanocomposites: *Recent Developments and Future Prospects*, Springer, England,1-44 (2016).

[16] K. Gupta, P. Jana, A. Meikap, *Synthetic Metals*, 160, 1566 (2010).

[17]R. Dou,Y. Shao,S. Li,B. In,M. Yang., *Polym.*, 83, 34(2016).

[18] P.K. Prabhakar, S. Raj, P. Anuradha, S.N. Sawant, M. Doble, *Colloids and Surfaces B: Biointerfaces*, 86,146 (2011).

[19]J. Konieczny,Z. Rdzawski,*Arch. Mater. Sci. Eng.*,56, 53(2012).

[20]G. Grass,C. Rensing,M. Solioz, *Appl. Environ. Microb*., 77, 1541(2011).

[21] M. Taner, N.Sayar, I.G. Yulug,S. Suzer, *J. Mat. Chem.*, 11, 13150 (2011).

[22]K. Dai,T. Peng,D. Ke,B. Wei, *Nanotechnology*., 20, 125603(2009).

[23] J. Zhang, H. Zou, Q. Qing, Y. Yang, Q. Li, Z. Liu, X.Guo, Z. Du, *J. Phys. Chem. B.*, 107, 3712(2003).

[24] W. Zhou,J. He,Sh. Cui,W. Gao, *Open. Mater. Sci. J*., 5, 51 (2011).

[25] S. Rezaee,M. R. Moghbeli,*Iran. J. Chem. Eng*., 11, 45(2014).

[26] L.Jin,Sh. Yang,Q. Tian,H. Wu,Y. Cai, *Mater. Chem. Phys*., 112, 977(2008).

[27] J. Siegel, O.Kvitek, P.Ulbrich,Z. Kolska,P. Slepicka,V. Svorcik,*Mater. Lett*. 89, 47 (2012).

[28] L. Pham,J. Sohn,J. Park, H. Kang,B. Lee, Kang, Y.Radiat. *Phys. Chem*., 80, 638 (2011).

[29] J.O. Noyce,H. Michels,C.W. Keevil, *J. Hosp. Infect*., 63, 289 (2006a).

[30] J.O. Noyce, H. Michels, C.W. Keevil, *Lett. Appl.Microbiol*., 49, 191(2009).