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Antibacterial activity and conductivity properties of composite nanofiber of cellulose acetate and Ag-Cu hybrid (bimetallic) nanoparticles

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Abstract: Hybrid nanoparticles structurally and physically differ from the single samples or non-nano and or bulk samples. In this work, the preparation of composite nanofibers has been run by electrospinning based on cellulose acetate and hybrid nanoparticles of Ag-Cu. The hybrid nanoparticles of Ag-Cu (Ag-Cu NPs) have been supplied through the reduction of their nitrate salts by sodium borohydride at various concentrations of Ag-Cu nanoparticles. The above nano-particles have been systematically detected by UV-Vis, EDAX, SEM and FT-IR. Considering the presence of Ag-Cu NPs in the nanocomposites, a remarkable conductivity has been obtained for the mentioned nanocomposites by four-point probe method. Also it has revealed asignificant antimicrobial activity against Staphylococcus aureus (gram-positive) and Escherichia Coli and Klebsiella pneumoniae (gram-negative)bacteria.

Keywords: Cellulose acetate, Nanocomposite, Cu-Ag nanoparticles, Antimicrobial activity, Conductive properties, Hybrid nanoparticle, Nanofiber.

Introduction

Hybrid nanoparticles have the structural and physical properties different from single samples or non-nano or bulk samples [1, 2].Hybrid or bimetallic nanoparticles [BMNP] have a wide variety of applications in technologies compared to mono-metalnanoparticles, from more robust antibacterial properties to optical features, energy saving, various applications in medicine such as quarantine, security, drugs and magnetic resonance imaging [3-7]. The most important method for composite nanofibers containing nanoparticles is electrospinning, both high in diversity and convenience [8]. Plenty of polymers have been produced through this method [9].

Because nanoparticles enhance nanofibers properties and composite nanofibers applicability is obtained [10, 11].

On the one hand,conductive polymer composites have attracted remarkable attention and represented some promising applications in many fields like energy saving, sensors, electromagnetic protection, corrosion, microelectronics and so on [12, 13]. Many of the modified conductive polymers and synthetic textiles composites can be employed in the emerging technologies such as electron load loss [14], electromagnetic protection [15], and scaffolding for engineering textiles [16-18]. For instance, Leo et al.,have prepared electric conductive cellulose films containing nanoparticles of gold[19]. Also, the bacterial adhesion and cell culture studies showed that the polymer/metal nanocomposites control the antibacterial activity and improve the biocompatibility compared to the virgin polymers [20]. According to the conducted studies, so far cellulose acetate nanofibers with copper/ silver hybrid nanoparticles

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(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 and silver nanoparticles through copper and silver salt reduction and following it, electrospinning of polymer solution has been supplied and its conductivity traits have been measured. Moreover, due to the presence of Cu and Ag nanoparticles, both being antibacterial, the antibacterial properties of the produced nanocomposites have been evaluated.

Results and Discussion

Fourier Transform Infrared Spectroscopy (FT-IR):

In the blank sample (nanofibers of cellulose acetate) demonstrated in the spectra of Figures **1** and **2** with letter **a**, there are two strong peaks in the area 1031 and 1686 cm^{-1}, indicating the acetyl group ,the absorption band 3591 cm^{-1} belongs to group H-O. Symmetric and asymmetric vibrations of CH_3 are spotted at 1323 and 1404 cm^{-1}[21-24] [Figure **1** spectrum **a**].Considering IR spectra of electrospun sample of blank fibers (nano Cu/Ag free) and composite nanofibers containing 300ppm concentration of Cu-Ag nanoparticle, 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 1046 and 1749 cm^{-1}, respectively and shifted with a CH_3 group peak related to the blank sample acetyl group from 1321 cm^{-1}to 1375 cm^{-1} in the sample of 300ppm copper. Besides, the peaks intensity has increased in the samples containing 300ppm nanoparticles (Figure **1**). Observing the samples spectra with the higher concentration of copper nanoparticles, i.e., 500, 1000, 1500 and 2000 ppm relative to blank spectrum, the mentioned index peaks intensity has increased significantly. The peaks shifting and their intensity rise imply nanocopper penetrating nanofibers and creating strong chemical interaction with it (Figure **2**).

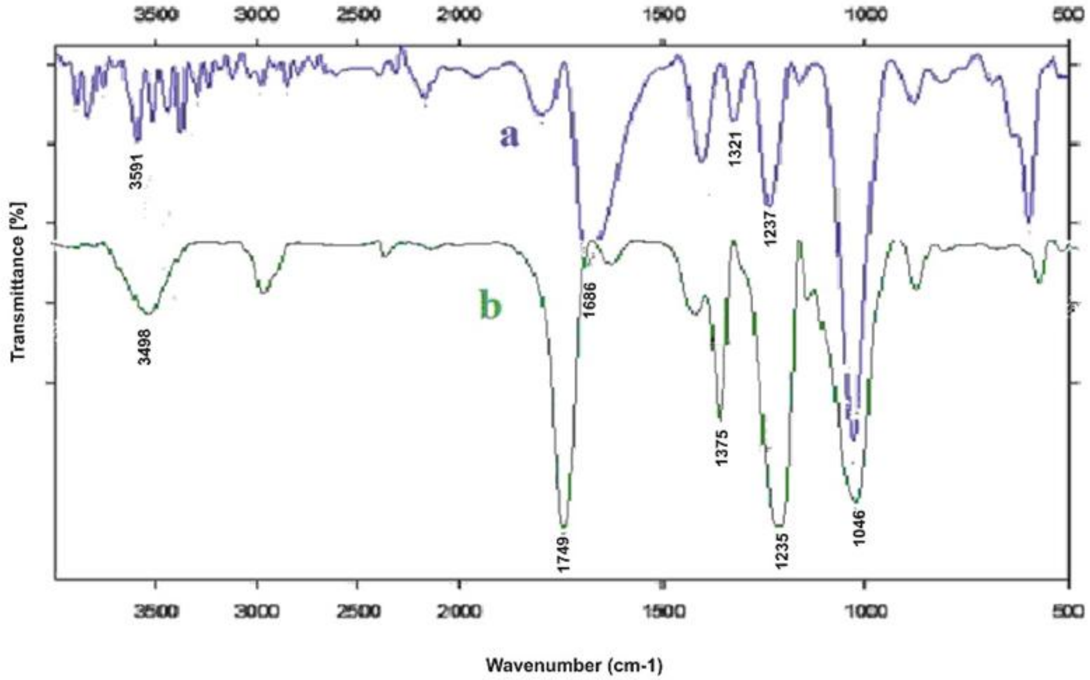


Figure 1: IR spectrum of composite nanofibers:(a) blank sample (electruspun nanofiber without nanoparticles); (b) composite nanofibers sample at concentration 2000ppm of Cu-Ag nanoparticles

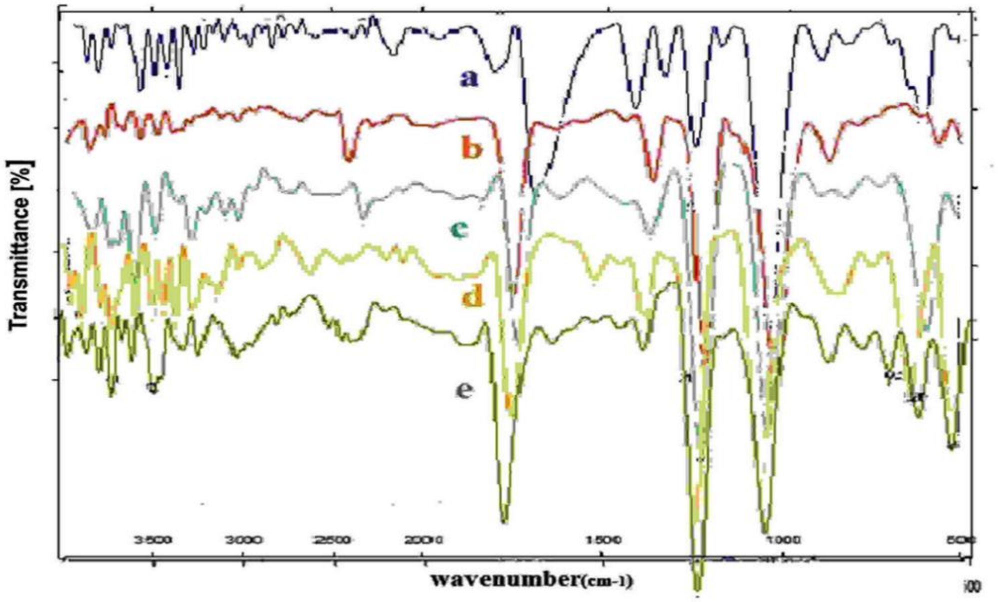


Figure 2: The in situ nanocomposite IR spectra: a) the blank sample & with Cu/Ag nanoparticles at concentration b) 300 ppm c) 500 ppm c) 1000 ppm d) 1500 ppm e)2000 ppm

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 silver and copper nanoparticles is optimally uniform. Besides, the size of Ag - Cu nanoparticles and their appropriate distribution is vivid in electron scanning images, as these images display the average size of nanoparticles varies from 30 to 50 nm (Figure **3**). 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 30-40nm while at concentration 2000 and 1000ppm, their average size gets bigger reaching around 40-50mm. This is because when nanoparticles concentration increases, their adhesion goes up and (Figure **3**).

UV -Vis Spectroscopy:

Silver and copper nanoparticles (Ag-Cu/NPs) production and size and also their relative distribution can be followed up and proved viaUV-Vis so that the absorption peaks in 550-600 nm area and surrounding 400 nm can be attributed to copper and silver nanoparticles, respectively [25, 26-28]. While there was no peak in this area before sodium borohydride increase. After adding, absorption peaks are observed in this area , which is the reason behind the formation of such nanoparticles. Moreover, regarding the maximum wavelength 525-555 and 423-433 nm for Cu-Ag nanoparticle respectively and maximum wavelength for 500 and 1500 ppm concentration ofsilver and copper nanoparticles indicate the small size about 30-50nm of these particles (Figure **4**).And ultimately, as nanoparticles concentration increases, the peaks intensity goes up, too [25, 29-32].

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

X-ray Diffraction Spectroscopy (EDS or EDX) used to detect the elements existing in a sample is based on this matter that an energetic ray of charged particles such as electrons and protons or an X-ray focuses on the study sample and removes the lower electrons such as the layer K and L and creates a cavity; as a result, the upper layers electrons fall in thiscavity and a lot of energy as much as X-rays is released. The number and amount of the released energy depends on the element used in the elemental analysis. Here, employing this spectroscopy, the presence of the elements copper and silver is perceived well (Figure **5**).

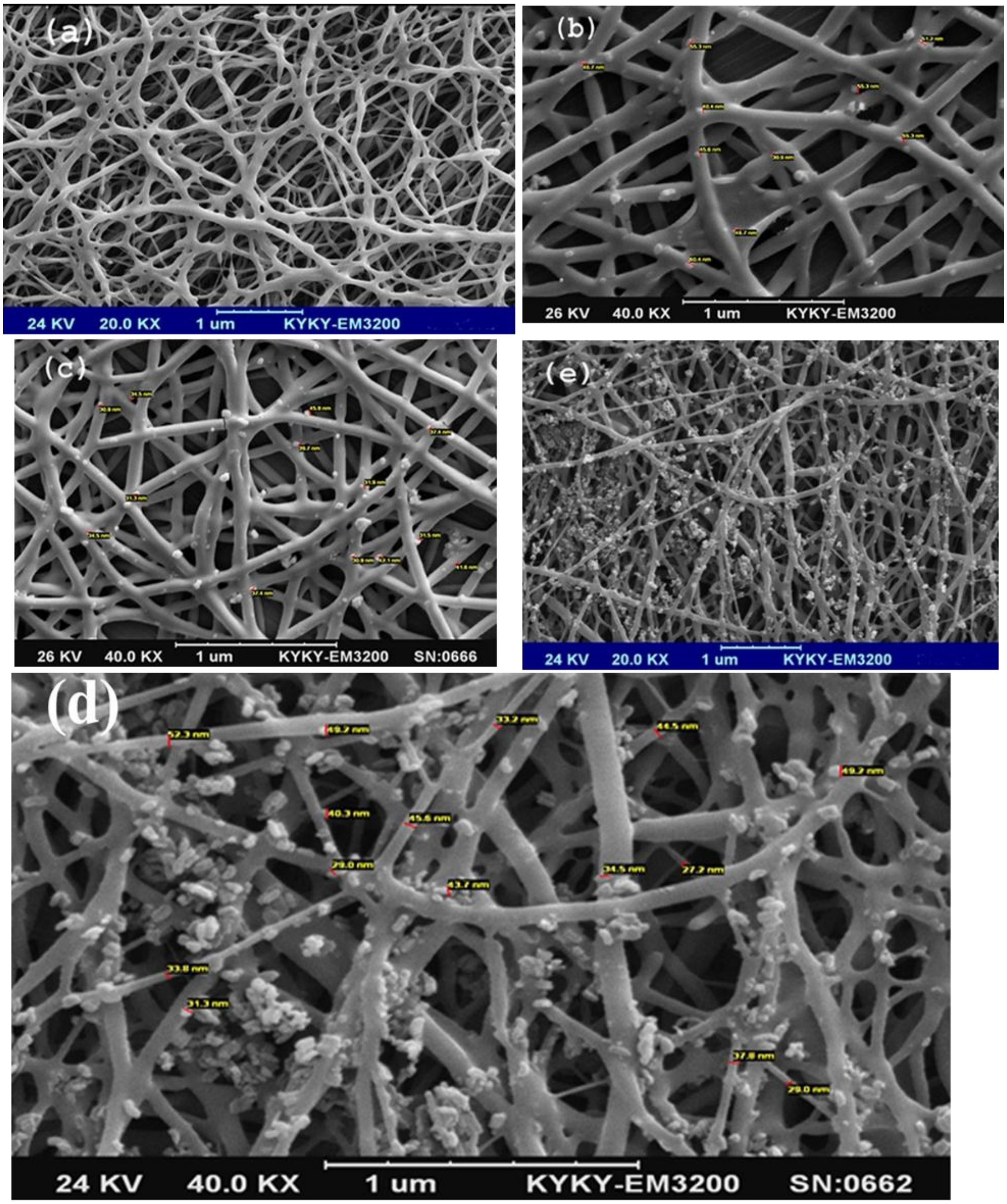


Figure 3: SEM image of nanocomposite sample at acetate celluloseconcentration 15% w/v(a) blank sample and at copper/silver nanoparticlesconcentration (b) 300 ppm (c) 500 ppm (d) 1500 ppm (e) 2000 ppm

Nanocomposite Conductivity:

The nanocomposites`conductivity results have been given in Table **1**. as depicted in Table **1**, the blank nanocomposite (Cu/Ag-Cu nanoparticles free sample) has no conductivity but the species having nanoparticles got conductive. Also the conductivity increases as the copper nanoparticles in the nanocomposite rise.

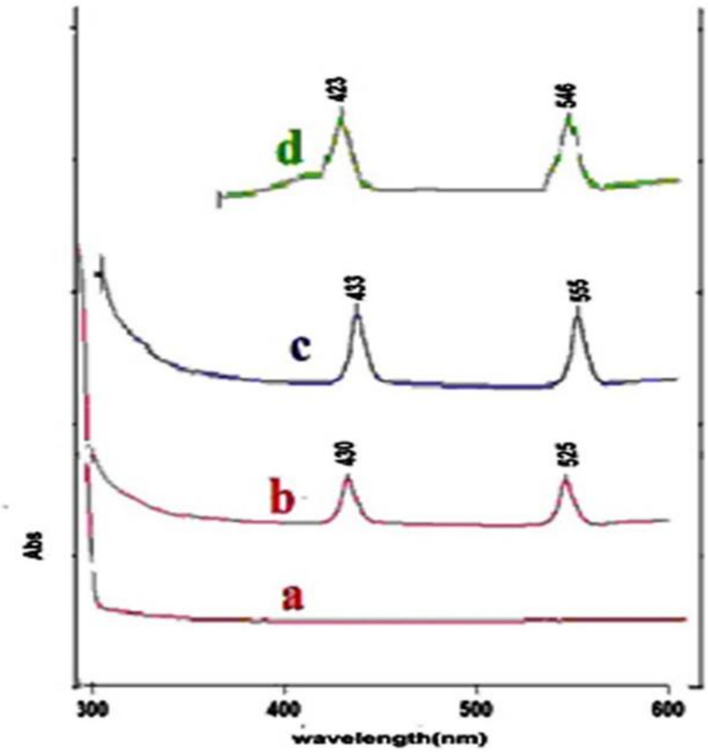
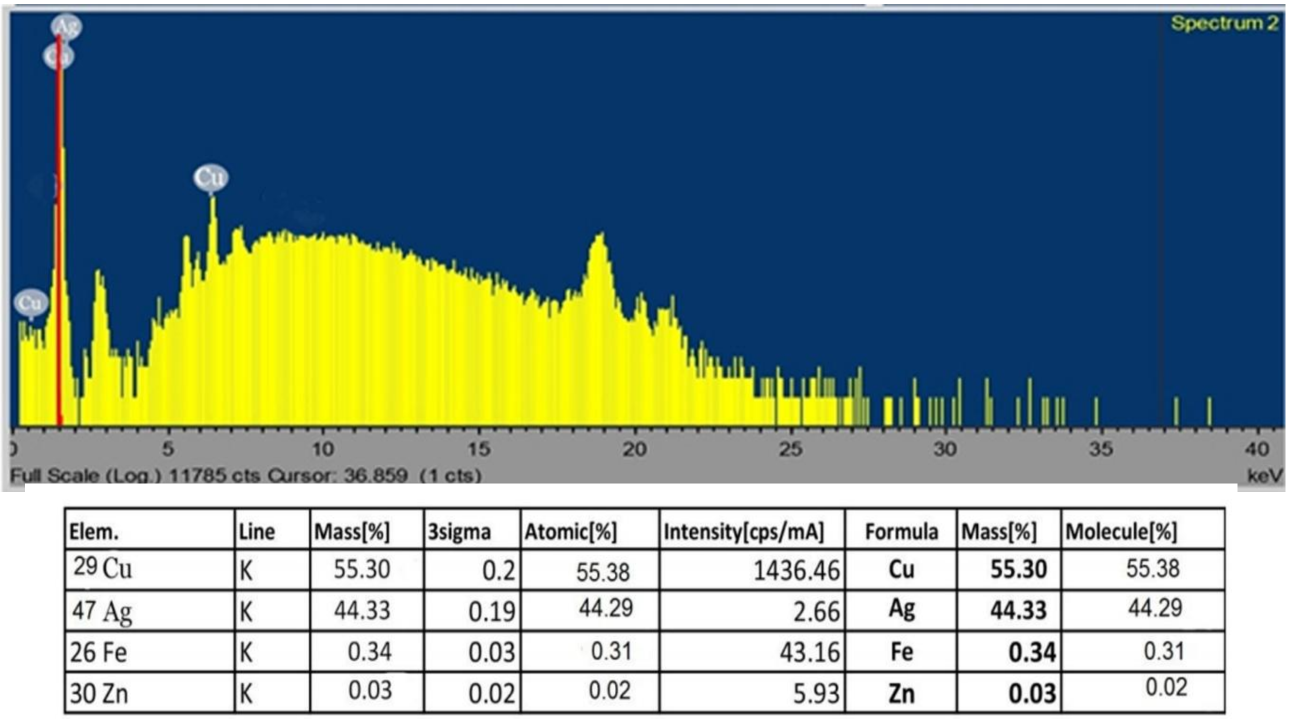


Figure 4: UV-VIS spectrum of composite nanofibers: a) blank sample (electruspun nanofiber without nanoparticle); b) with copper and silver nanoparticles at concentration;b) 500 ppm c) 1000 ppm d) 2000 ppm



Elem.	Line	Mass[%]	3sigma	Atomic[%]	Intensity[cps/mA]	Formula	Mass[%]	Molecule[%]
29 Cu	K	55.30	0.2	55.38	1436.46	**Cu**	**55.30**	55.38
47 Ag	K	44.33	0.19	44.29	2.66	**Ag**	**44.33**	44.29
26 Fe	K	0.34	0.03	0.31	43.16	**Fe**	**0.34**	0.31
30 Zn	K	0.03	0.02	0.02	5.93	**Zn**	**0.03**	0.02

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

Antimicrobial Tests:

Regarding this matter that the antibacterial property of silver is inherently stronger than that of copper. But 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 (25º C) and normal humidity (50%) is able to kill 99.9% of microbes, thus antibacterial test has been done with nanocomposites, yielding satisfactory results [33-36]. Therefore, the antibacterial properties have been performed with gram-positive bacteria as **Staphylococcus aureus**, and gram-negative ones such as **Escherichia Coli** and **Klebsiella pneumoniae**. The results have been obtained from 5 iterations showing the bacteria growth rate variation percentage. Considering the antibacterial tests results given in Table **2**. Regarding the antibacterial results,it has been determined that nanocomposite has significant antimicrobial properties and also, as Ag-Cu concentration increases in the nanocomposite, the antibactrial properties get more.

Table 1: comparing the synthesized nanocomposites conductivity

Entry	nanoparticles concentration in nanocomposites(ppm)	conductivity of nanocomposite(uS/cm)
1	·	·
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}

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%)	klebsiella pneumoniae percent reduction of colony (R%)
1	300	43	70	**64**
2	500	45	82	**72**
3	1000	49	85	**45**
4	1500	64	86	**69**
5	2000	69	90	**80**

Conclusions

nanocomposites including acetate cellulose containing CA/Ag-Cu NPs have been produced successfully by electrospinning .Ag-Cu nanoparticles have been yielded through reducing copper (II) nitrate and silver nitrate by sodium borohydride. Various tests have been employed to detect the above nanocomposites successfully. SEM images have displayed that Ag-Cunanoparticles have been uniformly distributed in nano-composites and in the sizes of 30-50nm. This supports Ultraviolet spectrometry, too. EDS spectrum of this composite exhibits Ag-Cu nanocomposites in composite nanofibers tissue. FT-IR spectrum shows the interaction between Ag and Cu nanoparticles sand nanofibers, so that the index peaks have undergone significant shift and the peaks intensity has changed, too. also, compared to acetate cellulose nanofibers,the above nanocompiste has exhibited good conductivity. And finally, its antibacterial properties are seen due to the presence of Ag-Cu nanoparticles against gram positive bacteria such as Staphylococcus***aureus*** and gram negative bacteria, namely, **Escherichiacoli** and**klebsiella pneumoniae**, promisingan antibacterial and conductive nanocomposite in future applications.

Experimental

Materials:

Copper(I) nitrate, silver nitrate and sodium borohydrate 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

elements existing in a nonocomposite .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**,** Equation**1** has been applied.

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

Equation 1:computing bacteria drop rate %R: percent Reduction of colony.

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. This manner that 250 mg $AgNO_3$. $3H_2O$ is dissolved in 100 ml of the solution including 70 ml Acetonitrile and 30 ml distilled water .Under severe stirring, 8 ml of sodium borohydride solution with the same above solvents ratio is added to the previous system drop by drop stirred under nitrogen gas and severe stirring conditions so that at the end, the solution color turns bright red .After being completely reduced, the solution is centrifuged and the nanoparticles are separated and washed with methanol and dried under vacuum [37].To prepare Ag nanoparticles, sodium borohydride and silver nitrate as 1:5 ratio have been used.This way that , in a solution of acetonitrile and ethanol solvents and DMF in 2:1:1 ratio,sodiumborohydride has been dissolved depending on the required concentration of Ag nanoparticle. After that 5 ml silver nitrate at certain concentration is added drop by drop and after the reaction getting over, it is totally homogenized via being stirred for 4h [38]. Then a certain amount of the resulted nanocopper is added to the above solution so that a mixture of Ag and Cu is supplied at a certain concentration.Following it, in a beaker at 60 °C, 375 g acetate cellulose is added to 25 ml of the same above solution ratio and placed in the shaker for 4 h and totally dissolved and homogenized. Later, the produced Cu-Ag nanoparticles solution in the previous stage as 1:1 ratio is added to the polymer solution. The above solution is placed in the Ultrasonic Homogenizer for an hour so that in addition to getting homogenized,it results in the probable nanoparticles binding completion with the polymer. This way that using the amount of the compounds used in the polymer solution at nanoparticle concentrations, the Ag-Cu nanoparticles have been prepared at the concentrations as 300, 500, 1000, 1500 and 2000 ppm. After that, the above solutions are converted into composite nanofibers using electrospinning device. This way that the aforementioned solution mixture in the device is added to a 5 ml syringe and 27 KV voltage is applied between the syringe needle and collector drum. The rate of the above solution feeding is set 0.5 ml/h.The distance between the syringe head and the drum is 15 cm.The drum rotation speed is set 2 cycles/min and the device environment temperature has been reported 25°C.

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