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RESEARCH ARTICLE

Preparation and Characterization of Bacterial Cellulose and its Composites with Nano Silver Particles of Bioactive Elements

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Abstract

Bacterial cellulose has unique physical and mechanical structural properties. Which qualifies it for use in many industrial, medical and other applications? It is more effective in putting out wounds or burns as it used as a catch to take advantage of one of its advantages, which is its ability to retain fluids for longer due to its low degree of crystallization. Where a study carried out for the preparation of Nano silver cellulose compound of two methods, namely thermal reduction and Electrical mothed. An FT-IR spectrum analysis was s and to know the interconnection between silver Nano particles with bacterial cellulose. The size of the particles was estimated silver nanoparticles of (34.6-35.0) nm respectively for both methods with the AFM technique and to ensure the occurrence of actual adsorption with UV-Vis, SEM and EDX technology, while the x-ray technology showed the shape of silver Nano particles cube and at a volume rate of (4.49, 10.06) nm respectively for both methods and was studied Biological efficacy towards the bacteria of wounds and burns where the results of both methods showed a strong effect to inhibit and stop these bacteria, Finally, compare it to both methods in terms of advantages and disadvantages in terms of the purity of the output was the best but the rate of molecular size and biological effectiveness and time of thermal reduction is the best.

Keywords: *Bacterial cellulose; Silver nanoparticles; Composites; Antibacterial activity.*

Introduction

Bacterial cellulose (BC) is a biopolymer, synthesized by microorganisms, similar in chemical structure to plant cellulose, but possessing unique physical-mechanical and chemical properties. BC is characterized by high biocompatibility, does not show cytotoxicity or allergic reactions. The BC demonstrates no adhesion towards opened soft tissues, so it can be an ideal material for the wound dressing [1]. Silver nanoparticles are nanoparticles of silver of between (1-100) nm in size [2]. Nano silver is not a new discovery; it has been known before it was for more than 100 years. Ag NPs or suspensions of Ag NPs mentioned to as colloidal silver. Before the discovery of penicillin in 1928, colloidal silver had been used to treat many contagion and diseases. By changing bulk silver into Nano sized silver, its effectiveness for controlling bacteria and viruses was increased, primarily because of the nanomaterials have extremely large surface to volume ratio compared to bulk silver, thus resulting in increased the area of contact with bacteria and fungi In 1951, Turkevich et al. reported a wet chemistry technique to synthesize Nano silver using silver nitrate as a silver ion source and sodium citrate as the reducing agent for the first time [3]. Recent advances in nonmaterial science in the last two decades have enabled scientists to control silver nonmaterial size and shape, which in turn control the chemical, physical and optical properties of Nano silver. The unique properties of silver nanoparticles have been exploited in a wide range of potential applications in medicine, cosmetics, renewable energies, environmental remediation and biomedical devices. According to the novel properties of Ag-NPs over 250 products containing Nano silver are now available for public use, this has made Nano silver the largest and fastest growing class of Ag-NPs in consumer products application [4]. Silver, either as nanoparticles

(Ag°), oxides (mainly Ag_2O), or in ionic forms (Ag^{+}) shows excellent antibacterial [5].Activity Nano silvers is widely used in various areas with exponentially increasing production. Apart from traditional usage in the engineering industry {e.g., catalysis, optical devices, surface Plasmon resonance (SPR), surface-enhanced Ramon spectroscopy (SERS) and electronic applications excellent antibacterial activity made them popular in a widespread range of applications (e.g., fabrics, disinfectants and medical devices). Nano silvers represent a broad spectrum of antimicrobial activity, and can kill both Gram-positive and Gram-negative bacteria (e.g., Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Staphylococcus aureus) [6].

It shown that Nano silver could inhibit the growth rate of bacteria from the initial contact with the pathogens, and had their antibacterial activity by killing bacteria rather than by the bacteriostatic mechanism. Nano silver also has antifungal activity. They could inhibit a series of ordinary fungal strains, including Aspergillus fumigatus, Mucor, Candida albicans, Candida globate, Candida tropical is, Saccharomyces cerevisiae, and Aspergillus fumigates [7]. BC contains a significant amount of surface hydroxyl groups, Ag+ ions could be easily attached to the BC Nano fibrils by chemical bonding, which could act as the seeds for the Ag reduction process. There are several reported methods to prepare Ag/BC nanocomposites, but most of them suffer from a prolonged synthesis process as well as need harmful chemicals that in turn become the source of impurities in the hybrid material itself. Photo chemical reduction under UV radiation is one of the most environment friendly and fast reduction procedure to produce Ag nanoparticles in the cellulosic matrix that has not been explored much yet [8]. The Ag metal has the capacity to attach to bacterial cell membranes, to enter and attack the respiratory chain of the cell, hence inducing the death of the bacteria [9]. Ag NPs have been added to a cellulose fibers and have shown successful antibacterial activity in my search I worked a new mothed to prepare Ag/BC nanocomposites the first method by Thermal reduction AgNO3, and the second mothed by electric analysis of silver Both methods have yielded impressive results Both are characterized by being cheap .easy to prepare and safety .our study include a comparative study between two methods , one of them is reduction of Nano silver of bacterial cellulose surface without using a reducing agent and the two method is an electrical of preparing Nano silver on the bacterial cellulose surface.

Materials and Instrumental

Molasses (commercial product), vinegar (commercial product), date (commercial product), silver nitrate (AgNO3) 99% purchased from BDH, sodium hydroxide (NaOH) Pure pellets were purchased From (HIMEDIA),and used without further modification, sodium hypochlorite chloride (NaOCl) solution (3-8%), (commercial product) two rode silver 99.99% purity, 1mm dimeter and height 12.5cm, Battery charger 1-12 V. DC made in Shanghai / China, Electric Heater- Sunny japan, (pH, TDS) meter from china product, F-TIR-600 Spectrometer, SIDCO England, AFM - NT-MDT, Matera, Russian Federation, Energy-dispersive X-ray Spectroscopy- TESCAN, Vega 111. Czech Republic, TESCAN Brno servo, UV-visible spectrophotometer, SHMADZU CORPATION- Japan,

Method

Preparation of bacterial Cellulose. It includes four basic steps as follows.The first step is to prepare it for vinegar bacterium where different samples brought to the vinegar bacterium called (mother of vinegar) locally manufactured from super market the (300) g fundi date as in Figure 1.

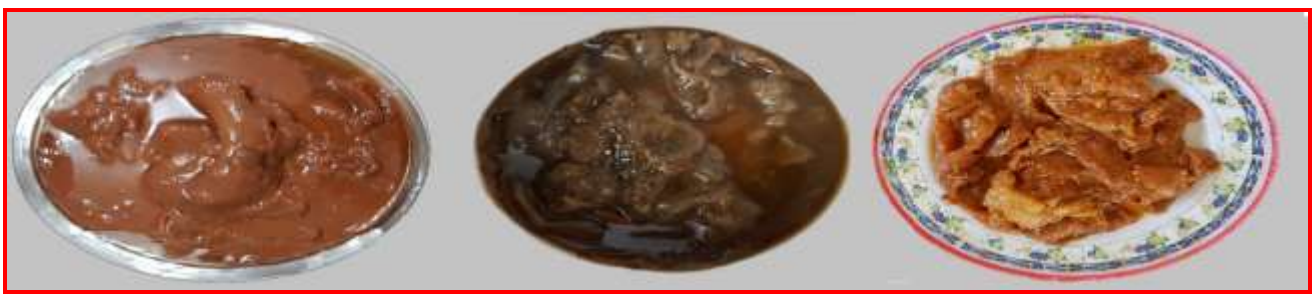

Fig.1: different shape of mother vinegar

On the other hand, prepare (500) g of the date juice (Al-Zuhdi), with 1L of distilled water the date washed for two hours in a bowl. Then, heating the dates at 70C° for five minutes after that, cooling immediately. The mixture were mixed by an electrical mixer at low speed for two minutes and other five minutes for maximum speed, the mixture were filtered with a clean cloth and the final volume is diluted with distilled water to a 40% wt/val. Date juice is ready to use for next steps. The second step: Increasing the amount of vinegar bacteria by cutting into 5 pieces each piece weigh 100 g.

The bacterial pieces distributed equally in a five plastic cans that are sterile and dry each with 1L capacity. Another mixture of date juice and ammonium acetate with (500ml-40% w/v and 5ml-0.1M) volume, concentration respectively, with 1L distill water as a diluent. The mixture then mixed well. Adding the mixture to the five cans evenly until the pieces of vinegar bacterial immersed. The cans closed precisely and kept in a dark place at 30C°for 4 days. At the end of each 4 days for 2 weeks Another mixture solution consist of date juice, ammonium acetate and distil water with the same previous concentration was prepared. Then, addition a 25ml of the mixture solution to each can with stirring. The third step: the fermentation phase of regular growth (at the end of the 2 weeks), this step involves isolating bacteria and increasing regular reproduction to create a thin bacterial cellulose that has equal thickness. Aggregation of *Acetobacter xylene* from the plastic cans in the second step in one bowl and mixed well in electrical mixer for one minute at slow speed and five minutes at maximum speed.

Then, poured into the same five plastic cans, which must be sterile, clean and dry. As referred earlier. The distributed mixture were very thin layer of bacteria in each plastic can. A 200 ml date juice added for each can and closed tightly. The cans kept in dark place at 30°C inside an electric incubator for (2-3) weeks. At the end of each three days, A 5 ml of date juice 40% w/v added to the cans that tightly closed immediately. Then, kept in the incubator. The growth of a regular-sized slice of bacterial cellulose after 3 weeks, as in Figure (2).

Fig.2: regular-growth bacterial cellulose slide

The fourth step: the isolation and bleaching the bacterial cellulose. The bacterial colony transferred to a carefully cleaned container. The containers washed with distilled water 3 times. Then, flooded with (NaOCl) solution for two hours. In order to get rid of yeasts, suspension of sediments and bacteria in cellulose tissue. As well as to whiten the cellulose tissue. Then, wash with distilled water to get rid of (NaOCl) solution. The containers washed about 6 times and repeat this process for 2to3 times until get white slice and moderate pH. After that, the bacterial cellulose is kept flooded in distilled water, to be ready for culturing and measurement, as in Figure 3.

Fig.3: bacterial cellulose bleached ready to use of later steps

A 10cm diameter piece of the ready cellulose obtained with the aid of a knife. The cellulose piece placed in a measuring tube filled with distilled water. Then, placed in a centrifuge for 5 minutes at low speed and for 4 minutes at maximum speed. After that, removal of water and replaced with another distilled water. The last step repeated five times to get rid of chlorine remnant or any other metal stuck.

Preparation of Ag NPs / B.C compound where it was prepared in two methods

Thermal Reduction Method

In this method do not use a reduced agent or any catalysts where a piece of cellulose prepared by the paragraph previously in baker or a test tube, flooded with distilled water and heated at about 80 C° for 5 minutes to get rid of the dissolved oxygen, lift the cellulose piece by metal tongs, put in Baker is dry, clean and is flooded with AgNO3 (300ppm). Cover tightly and put in a completely dark container for about two hours. Then heat the mixture at 70C° about 10 minutes until the color of the cellulose piece changes to brown repeats the same steps above on different concentrations of silver nitrate which is (500.700.1000) ppm. As Figure (3).

Figure 3: different sample of Ag NPs/B.C prepared by thermal reduction method

Electrical Method

This is the intercalation of silver nanoparticles on the surface of bacterial cellulose and is carried out in these following steps:

- Connects two silver poles to a wire attached to an electric current type D.C (car battery charger) equipped with a card of (1-12 volts) where they are placed a 200 ml glass baker filled with distilled water (Figure 4).The salinity is measured by the TDS. And moderate pH from the first minute beginning of the interaction and Repeat the measurement with a periodic process every 60 minutes (Table 1). Where we note that at the beginning of the interaction is slow interaction then the speed begins to increase and must clean the electrodes well whenever they turn to black color as a result of the oxidation of the surface of the pole and avoid stopping the interaction. In other hand, put a small piece of bacterial cellulose in a baker filled with 50 ml distilled water and heat the baker until it is boiling for (5-10) minutes until we get rid of the dissolved oxygen. Mix the cellulose piece with the Nano silver solution prepared in the electric way, where there are two successful ways to complete the interaction, namely. a-Heats the mixture for 10 minutes and at 70C° and then closes the baker cover tightly and put in a container dark for the purpose of making the required measurements.

- Close the baker nozzle tightly and put the baker in a dark container and after 24 hours heat the baker for 10 minutes at 40°C then close the baker tightly then put the container dark and close tightly for the purpose of making the required measurements (Figure 4, 5).

Table 1: Steps formation Nano silver by electrochemical method

Time at 25 °C, and volume 200 ml (hour)	Concentration PPM	pH	Color
0	**2**	6.9	colorless
1	**4**	7	colorless
2	**6**	7.3	colorless
3	**12**	7.5	Colorless
4	**14**	8	fuzzed
5	**17**	8.5	Few Yellow pale
6	**22**	9	Pale yellow

Fig.4: different samples of Ag NPs/B.C was prepare by electric mothed color changed a. after 12 hour's b . After > 12 hours

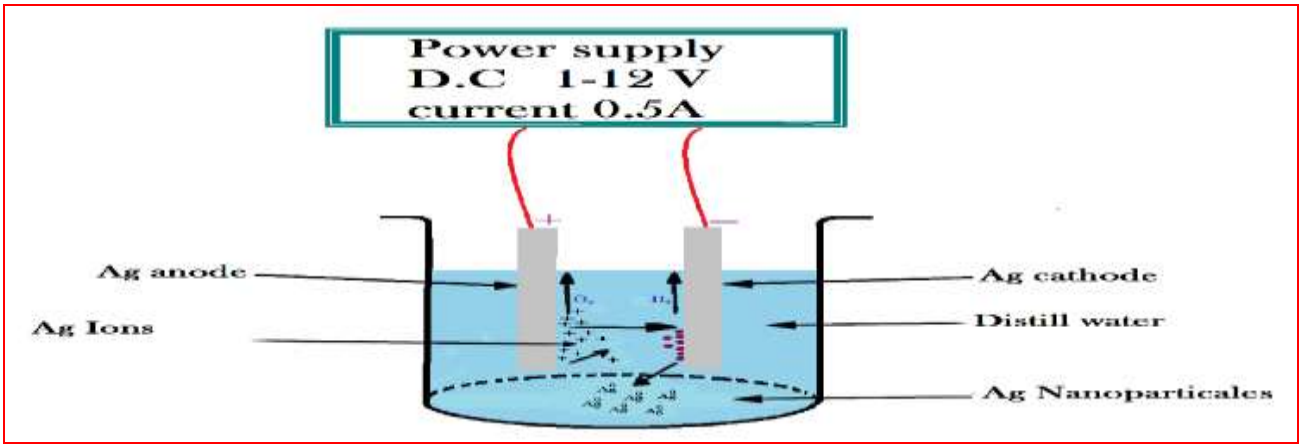

Fig. 4: samples of Nano silver prepared by electric method

In Table one show a direct proportion between time and concentration of Nano silver that prepared by the electrical method at fixed voltage. It was observed that the solution pH be more basic with time increase. Reduction of silver ion that formed in the solution on the cathode surface can cause increase in solution p H. In addition, it was noticed at 60C° the BC color be light yellow brown this indicate increase in Nano silver particles in the solution. The unique structure of BC and the presence of hydroxyl groups in cellulose fibers constitute an effective Nano reactor for *in situ* synthesis of the NPs. The ether and hydroxyl functions not only anchor the metal ions tightly onto the fibers via ion–dipole interactions, but also after reduction stabilizes the as prepared NPs via surface interactions.

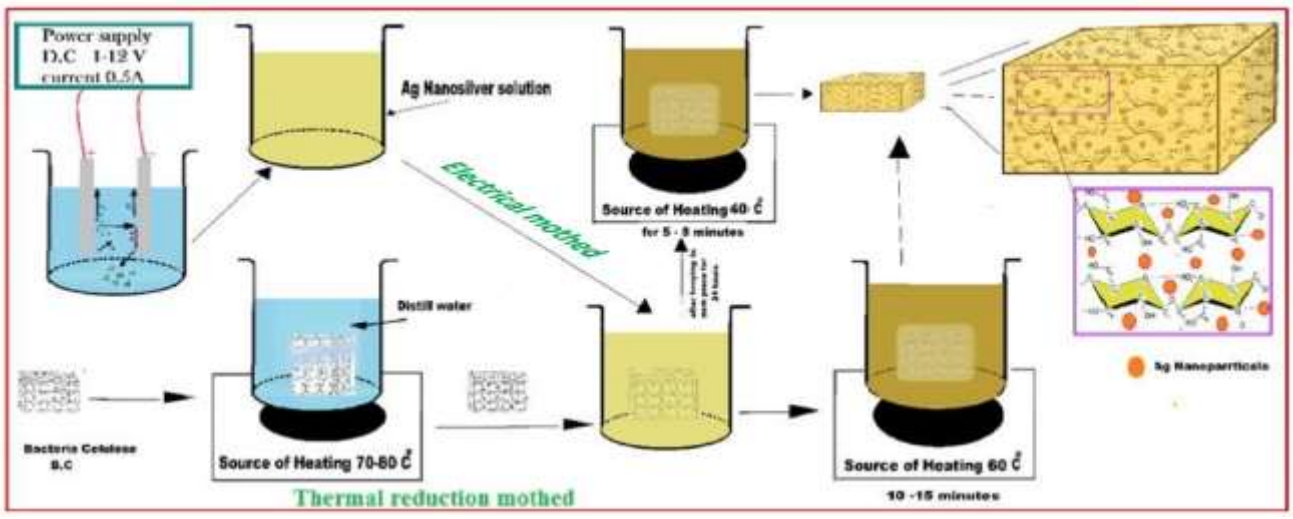

Fig. 5: In briefly, the preparation of a Nano silver compound on a bacterial cellulose in the thermal reduction and Electrical mothed methods

Results and Discussion

As a result of containing the composition of cellulose on functional groups such as, hydroxyl, and carbonyl, which act as a supporting anchor for silver ions absorbance on cellulose tissue, (Figure6) it is very easy to adsorption, due to their small Nano sizes compared to the size porous of the bacterial cellulose nanoparticles, which prevents the entry of Other bodies to the tissue. So the discoloration is the first proof of synthesis Ag NPs/ B.C on the other hand, The need to take into account the effect of several factors namely, temperature, concentration and time that are directly proportional to the occurrence of clusters or accumulations of silver on the bacterial cellulose tissue and to avoid this was used the lowest concentration of AgNO3(300)ppm, excluding the results of other concentrations, and the lowest possible temperature for the occurrence of Interaction (40-70) in as little time as possible, i.e. by color changed of cellulose tissue to pale yellow, the reaction ends immediately.

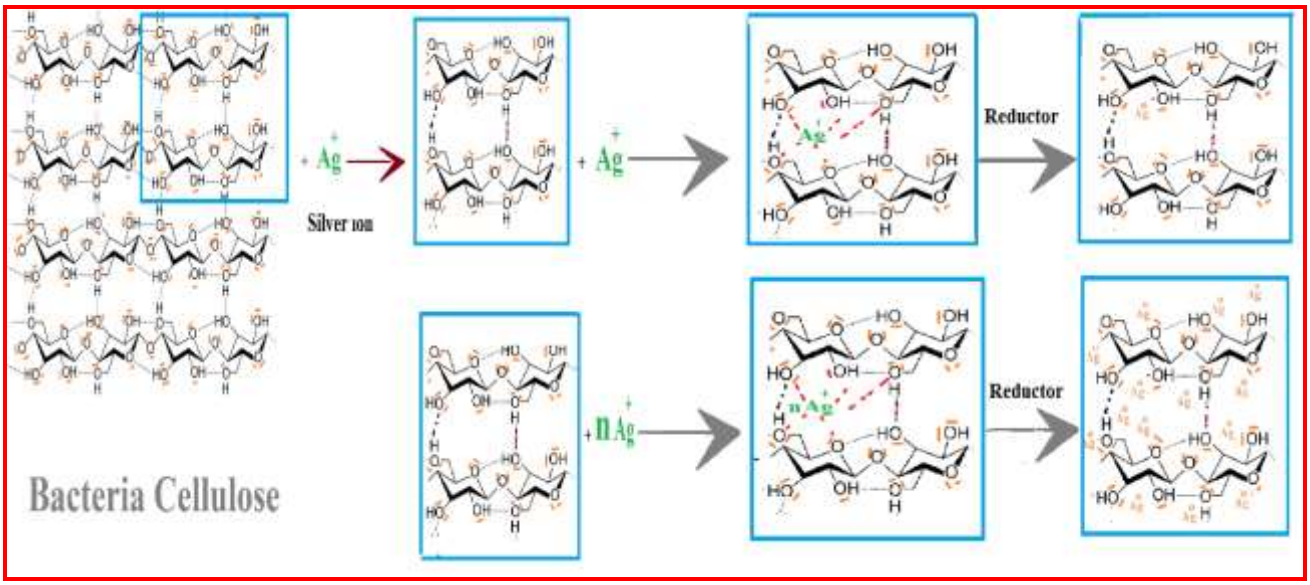

Fig. 6: adsorption Nano silver partials on the cellulose tissue

Interpretation of the FT-IR spectrum can explain in short summary as follows pure cellulose bacteria hydroxyl bond ingested by a large broad peak stretching (3200-3600) cm-1 appeared on a single peak at a frequency of 3421 cm-1 this is evidence of the strong correlation between intra and inter hydrogen bonds in pure cellulose. On other hand, spectrum of the Ag NPS/B.C compound that came in the first way (thermal reduction). We observe a change in the size and shape of the hydrogen hydroxide bond and the displacement of values from 3700 cm-1 to several values: (3732, 3460, 3483, and 3313) cm-1.

This indicates that the bonding of silver molecules with the inter-hydrogen bonds is the result of the displacement of the interconnections to the red shift, i.e. they have become larger because of their association with silver Nano particles. As for the spectrum of the Ag NPs /B.C compound, which was prepare in the second method (electric), we notice the appearance of one wide peak which is 3433 cm-1 within the range (3100 - 3600) cm-1 this is on two possibilities either the displacement of the underlying hydrogen bond towards the red shift , as a result of its association with silver, for this appeared as a single peak as a result of the convergence of its values extreme With the implicit association and the correlation of silver ions with the intra hydrogen bond connections and implicit [10].And the second probability is the most preferred and this indicates the efficiency of this method compared to the previous method. also notes the values of the C=O group at the frequency of 1743cm-1, which appeared clearly as a strong peak in the FT-IR spectrum of pure cellulose prepared laboratory.

As spectrum of the Ag NPs/B.C compound in the method of thermal reduction, we note a displacement in the wave number to (1664) cm-1 i.e. red displacement as a result of the weakness of its bond (C=O) as a result of the formation of a molecular bond with the surface silver atoms (or expressed by a complex formation with silver) which ferments In turn, the surface of the Ag NPs [10].

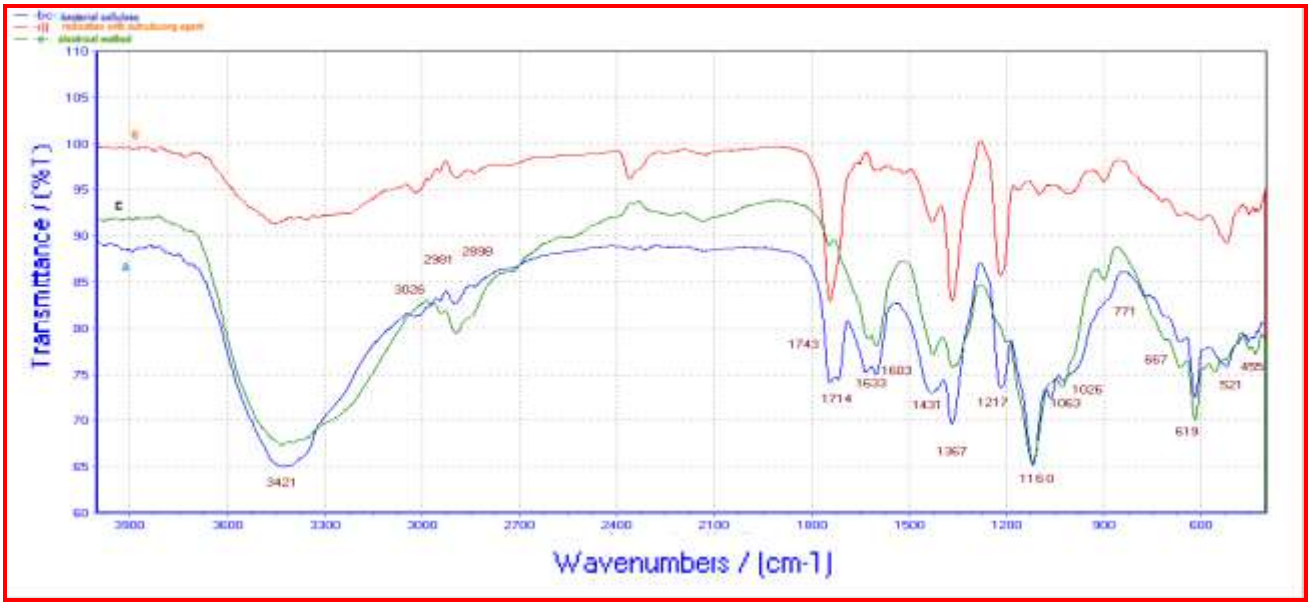

Fig. 7: FT-IR spectra for three sample a-Bacteria cellulose b- AgNPs /B/C by electric mothed C-Ag NPs /B.C by thermal reduction

Antimicrobial Activity Studies

The biological efficacy of silver Nano synthetic compounds adsorbenting on bacterial cellulose tissue prepared for both methods towards the bacteria of wounds and burns. has been studied negative gram and positive gram, we have observed results with the effect of bactericidal, or stop their action as shown in the farms testing the pathogens that have Negative effect on all cells of the body's tissues. A noted a difference in the control of the germs below can be explained due to the different physiological characteristics of the strains, which cause a different sensitivity to direct contact with silver ions. Silver nanoparticles are known to have cellular toxic activity towards viruses and pathogens, depending on the size and concentration of silver nanoparticles [16]. This enhances our work by emphasizing the adsorption Ag NPs on the cellulose fabric in both methods.

Table 3: Diameter of the zones of bacterial growth inhibition by composite BC films with silver nanoparticles obtained

Organism's	Medium of Mueller Hinton Agar at 37C°of 24 hours	
	Ag NPs /B.C by	
	Thermal Reduction / Diameter of inhibition zones(mm)	Electrochemistry method / Diameter of inhibition zones (mm)
E.coli	23	7
S. aurous	25	17
P. aeruginosa	30	/
Bacillus subtilis	23	20
Streptococcus	23	5
Acinetobacter Bauman nil	25	/
Klebsiella	28	7

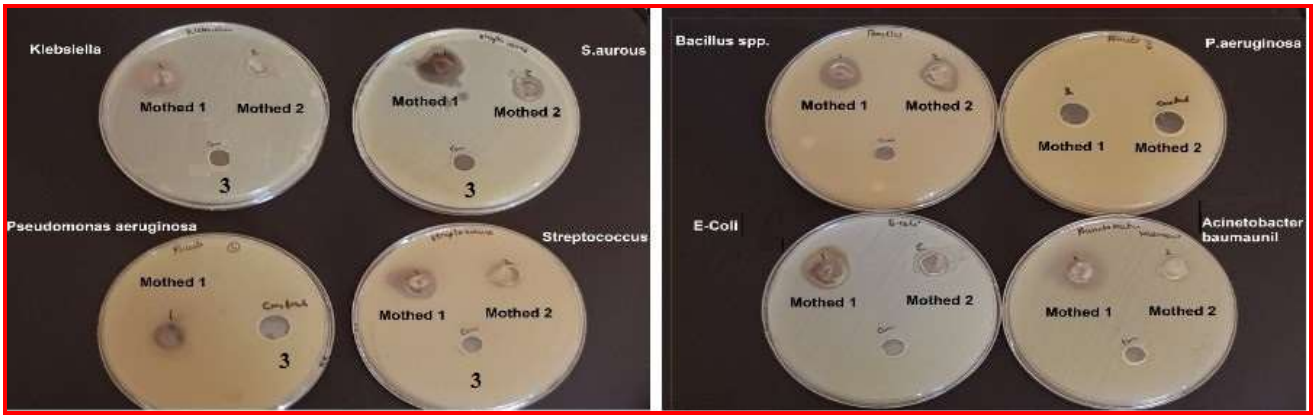

Fig.8: All Dose-dependent antibacterial activity of biologically synthesized AgNPs in Escherichia *coli, Klebsiella pneumoniae, Bacillus* spp, *Staphylococcus aureus,* Pseudomonas *aeruginosa* with the diffusion test with: 1- AgNPs/B.C by thermal reduction 2- Ag NPs/B.C by electrical mothed 3- pure bacteria cellulose

UV-Visible spectroscopy Nanocoient silver samples are absorbed at special frequencies resulting from the effects of surface plasmosis by the external oscillator electric field of the photovoltaic wave (surface plasma resonance) we note in the previous form of the measured folds the presence of only one absorption peak within the range (350-450) which is a peak characterized by resonance Plasma is a surface of silver spherical nanoparticles, and this proves that our samples are made up of spherical nanoparticles [17, 18].

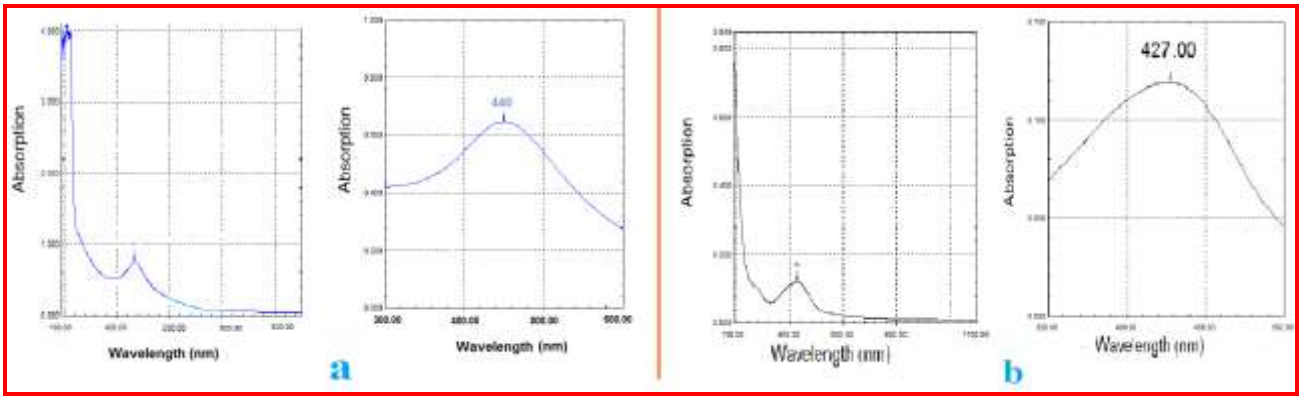

Fig.9: UV-Vis Spectroscopy of Ag NPs Composite: a-by thermal reduction, b- by Electrical mothed

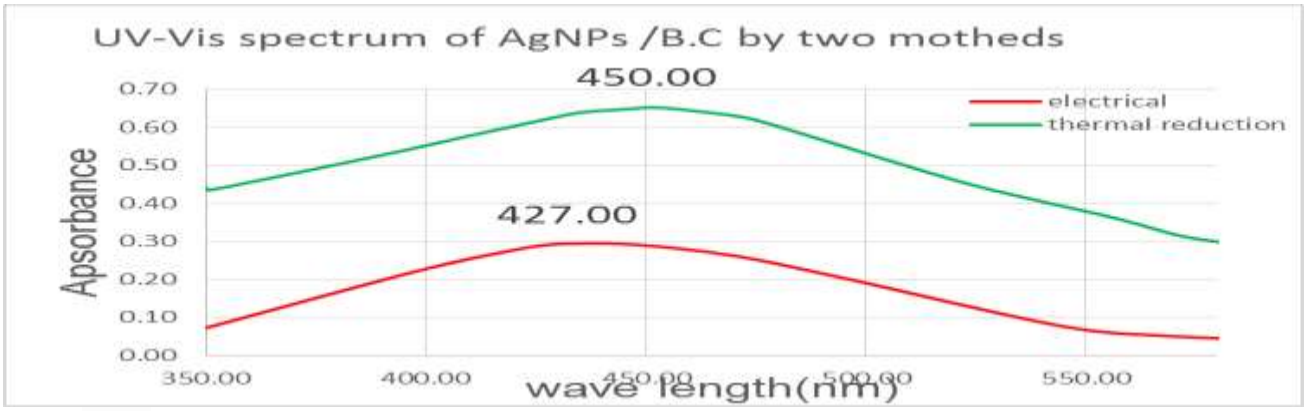

Fig.10: λ max of UV-Vis spectrum Ag NPs for two mothed methods (427-450) as a result of a large particle size further red-shifted the spectrum peak[19]

The crystallization of B.C and Ag NPs/B.C composite was calculated in x-ray diffraction dried the samples in a completely vacuum dry for 24-48 hours. From the Figure (11) notice the presence of two peaks at 14° ,22° degrees which represents (101, 002) respectively, these belong to bacterial cellulose and this proves the lack of crystallization and therefore its effectiveness is high [31]. The three peaks at values of (38.30º, 64.60º, 77.80 º) and (38.28º, 64.64º, 78.37 º) respectively, which correspond to the (111), (200) and (220) crystalline planes, there's Match with the customary Bragg reflections of face centered cubic (FCC) silver and confirm.

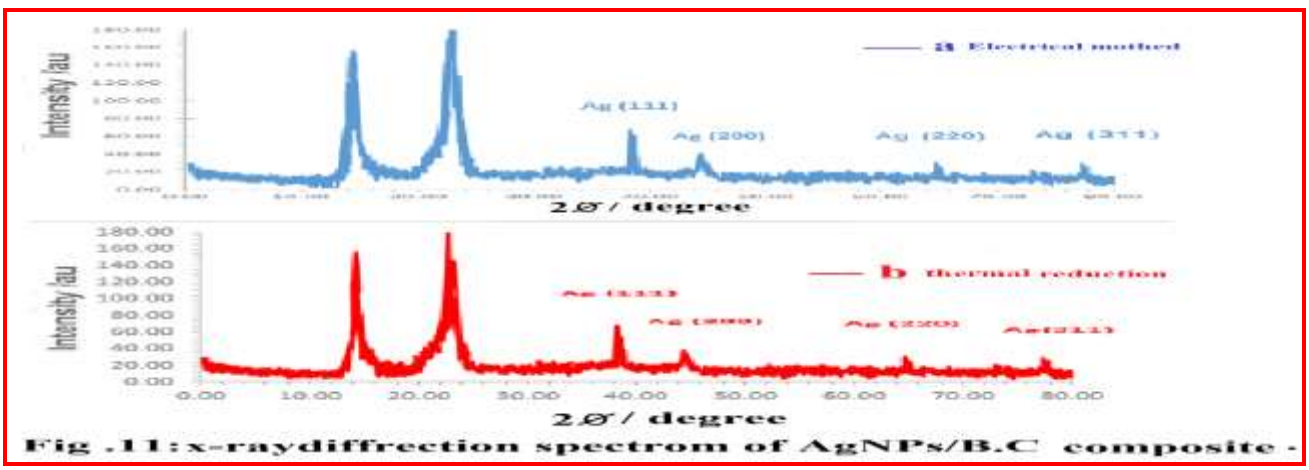

Fig .11: x-raydiffrection spectrom of AgNPs/B.C composite -

The analysis of the X-ray EDS. To determine the initial compositions of the models, has revealed the spectrum of the first model in (figure 12-a), of the presence of carbon and oxygen as a main percentage 42% and 40% respectively and return to the structure of cellulose as well as silver15%, which is the amount of silver in the first way in addition to the small amounts of chlorine and For sodium. This indicates the stability of silver particles on the surface of cellulose tissue with the presence of other ions [27]. As for the second method as in (figure 12 -b)and electrolytic we note the increase of the particles of neo-silver compared to the first method this indicates the increased stabilization of silver particles on the tissue of cellulose and the reduced percentage of other ions, namely chlorine and sodium, and this is evidence of the purity of this method.

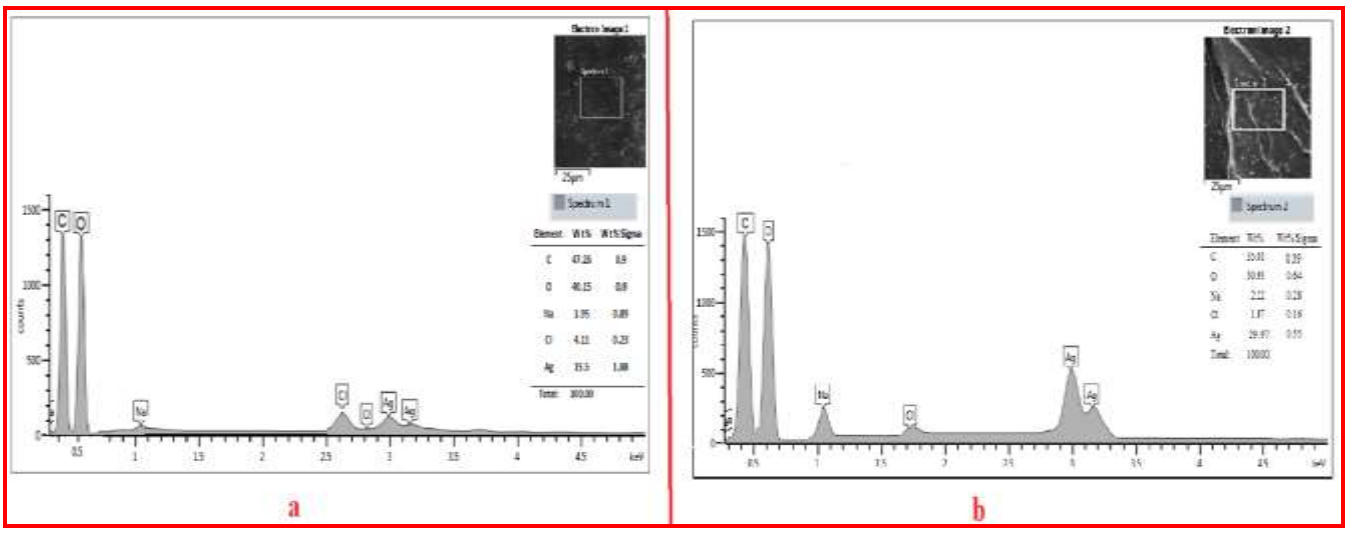

Fig.12: EDs of Ag NPs /B.C a- by thermal mothed b- by electrical mothed

Surface Morphology

The surface morphology of the Ag NP is studied through Atomic Force Microscope technique as Shown in figure (5).The AFM image of the surface morphology of the Ag NPs gives a good Indicator that particles are spherical in shapes and grains are tightly packed. The average particle size Determined from AFM, is about 34.625nm, and35.06 respectively of two methods.

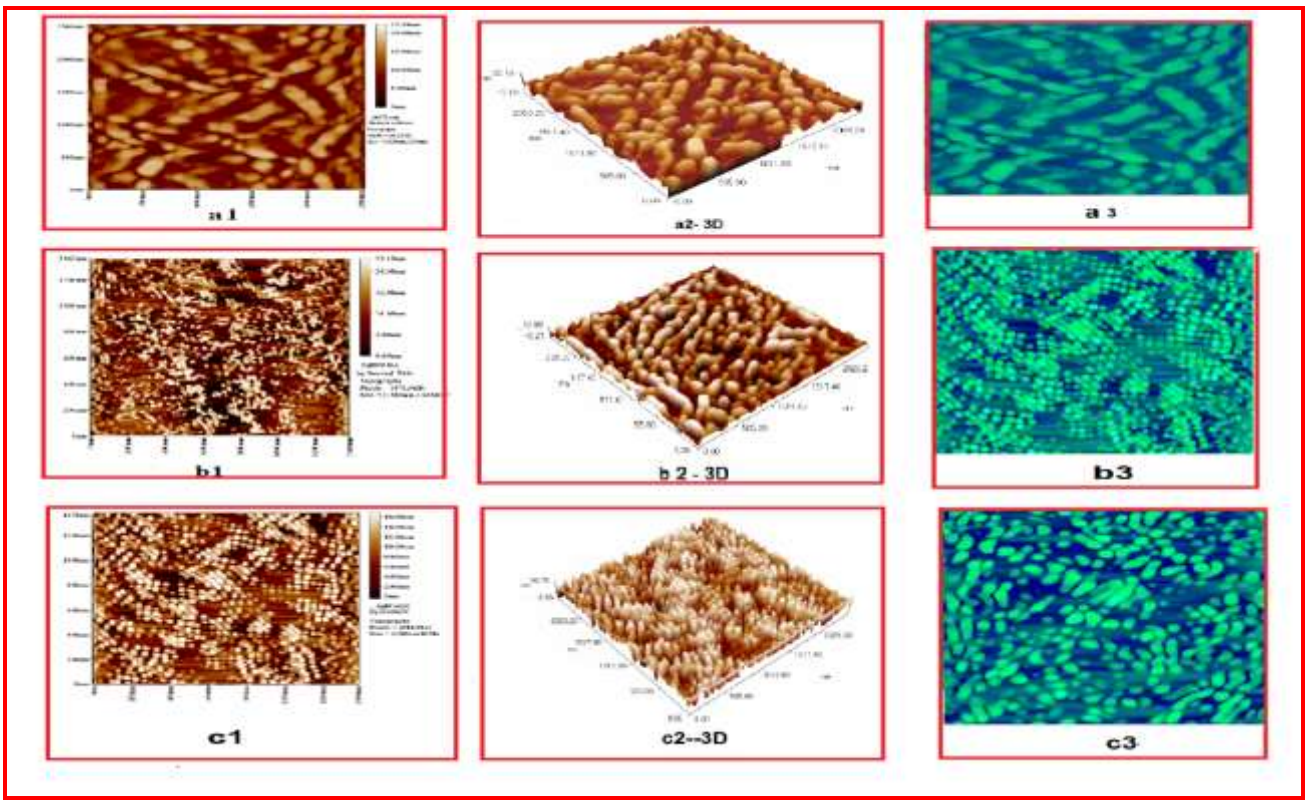

Fig.13: AFM pictures of substrates: a –Bacterial Cellulose: b- Ag NPs/B.C composite by thermal reduction: c- Ag NPs/B.C composite by Electrical mothed: 1-size topography mode, 2-3D photo three dimensions

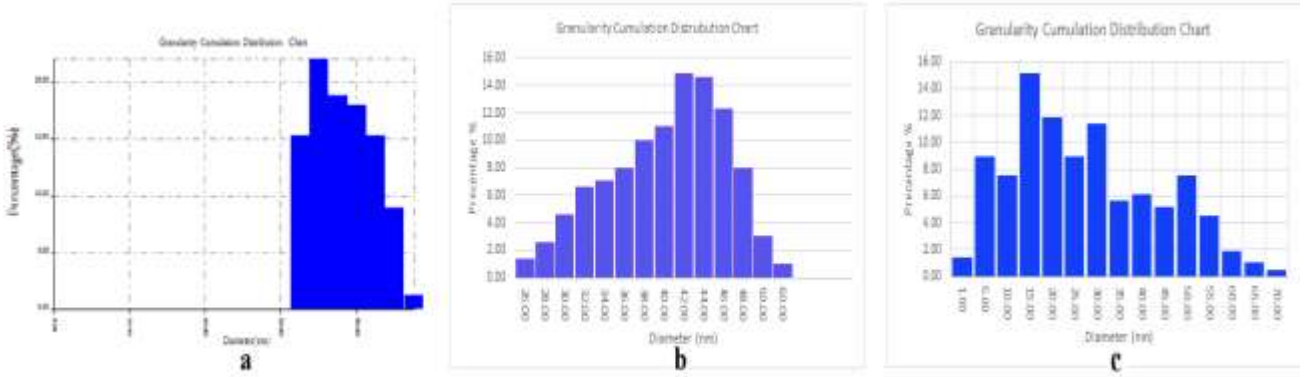

Fig.14: Histogram of a- Bacterial Cellulose average Pore size dimeter 74nm. b- AgNPs /B.C thermal reduction average dimeter 34.625 nm, c- electrical mothed average diameter 35.06 nm

Scanning Electron Microscopy (SEM)

Morphology analyses are contained in Figure 12- a, which display images consisting of porous fibers with a diameter of 0 to 70 nm in the form of 3D images of cellulose tissue. Accordingly, the distribution of fibers is in nanometers sizes. We note from the results of the images of scanning electron microscope as (figure15- a), bacterial cellulose consisting of a ultrafine 3D network, where the fiber is distributed in Nano dimensions of infinite precision interrelated so that form a woven structure that, this property helps silver spread by the spongy structure within the network Bacterial cellulose is evenly distributed through fiber and along the cellulose network either in the form of individual molecules or accumulations within the bacterial cellulose matrix and this is clearly visible from the images of (figure 15- b , c).Where silver appears in the form of clear shiny spots of the eye resulting from the recording of the image of the backing scattering electron, because it contains a much more atomic number than other components(C,O).we also note most of the molecules connected to the fibers while others are trapped inside the matrix [20].

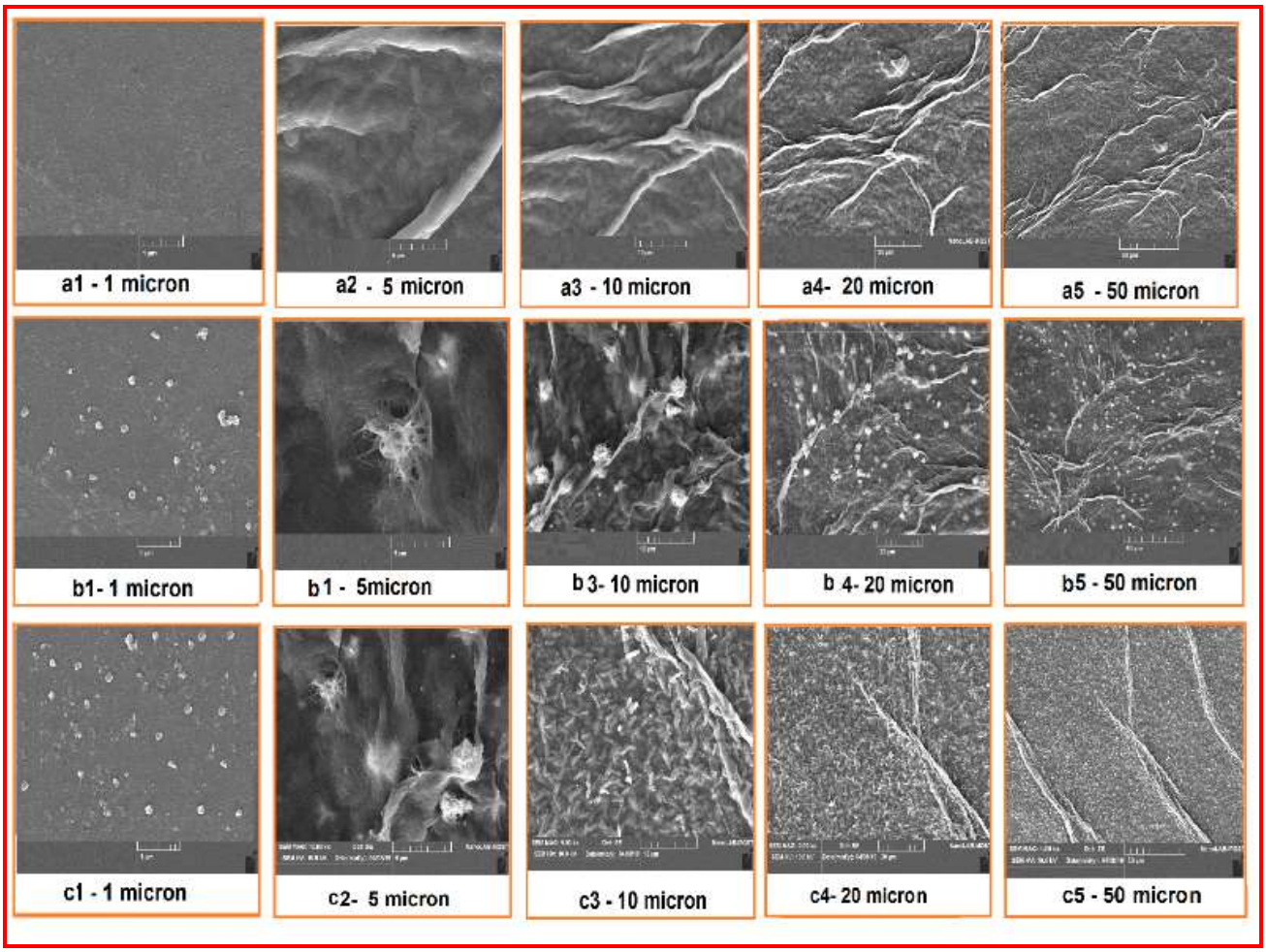

Fig. 15: SEM micrographs of the(a)pure bacterial cellulose, (b) Ag NPs/B.C composite by thermal reduction ,(c)Ag NPs composite by electrical mothed

Table 3: Compare between Ag NPs/B.C was prepared by thermal reduction and Electrical mothed.

NO.		Thermal Reduction Nano silver	Interjection Nano silver Electrical mothed
1	Time	Short about 2.15 hours	More approximately12 hours
2	Efficiency	More	Less
3	Rate	More	Less
4	Method	Easy	Novel
5	The cost material	Less	More
6	Purity	Less of mothed two	high
7	Average size Nano particle's	34.625	35.06
8	λ max (nm)	440	427
9	Linked silver with OH cellulose	Most particle's near with inter hydrogen bond	Most particle's near with intra hydrogen bond
10	Shape particle	sphere	sphere

Conclusions

Reduction and intercalation of surface cellulose bacteria the most important methods for preparation of Silver nanoparticles and its experimental set up is very simple, less costly and work at room temperature without any heat exchanger, vacuum equipment and gas handling equipment. The prepared silver nanoparticles were characterized using UV-Vis absorption measurement, and Atomic Force Microscope. UV-Visible shows the absorption peak at 424-440 nm respectively belongs to silver nanoparticles. The average particle size found to 35, n m for silver nanoparticles obtained by Atomic Force Microscope measurement.

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