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NanoCrystals form of Cellulose-ZnO-Ag composite production, TEM description and microbial sensitivity

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Abstract : Green nonmaterial is a term called for nanocrystals prepared from bacterial cellulose depending on easy production without hazardous chemical treatment and renewable nature. In our study, we use a carboxymethyl cellulase enzyme from *Aspergillus niger* for preparation of bacterial cellulose nanocrystales (CNCs). Also, the synthesis of ZnO-Ag heterostructure nanoparticals was done by using CNCs as stabilizing agent and forming CNCs-ZnO-Ag composites. The size and shape of CNCs-ZnO-Ag composite was studied using transmission electron microscope (TEM) with average size of 6-50 nm and the shape was optic rounded and oval. Finally, These CNCs-ZnO-Ag composite have been examined for their antimicrobial activity using various pathogenic microorganisms and recorded highly activity.
Keywords : Cellulose NanoCrystals, Carboxymethyl cellulase, TEM, Antimicrobial activity.

Introduction

The acid hydrolysis of cellulose resulting in a composite called the cellulose nanocrystal (CNCs). Cellulose NanoCrystals (CNCs) have a lot of advantage because of its potential applications such as biomedicine[1], photocatalysis[2,3,4] and nanodevices[5]. CNCs have a lot of properties like high ability for intake of cations and gust molecules and perfect mechanical properties[6]. CNCs have many usages in material sciences such as polymers reinforcement, although it has low thermal stability [7]. So, to promote the thermal properties of CNCs we must combine it with inorganic nanoparticals.

ZnO-Ag has received great attention due to ZnO can be synthesis by simple process[8] and Ag nanoparticles have a perfect physicochemical properties. ZnO nanoparticles can inhibit both Gram-positive and negative pathogenic bacteria[9]. Also, the ionization energy of acceptors in ZnO is reduced by Ag^+ lead to emission enhanced[10]. So, Ag+ ions promote ZnO antimicrobial activity[11,10,12]. Contemporary, the serious potential applications of nanofibular materials as a carrier to make metallic nanoparticles in the synthesis of optoelectronics, catalysis, biomedical, sensors and electronic nanodevices have been gained great attention [13]. Different from the traditional stabilizers, the embedded particles and nanofubular size are in the range of nanometer, therefore, the characteristic large surface of nanoparticals and nanofibular stabilizer is preserve [14]. Moreover, these composites have the feature of inorganic particles like thermal stability, exceptional functionality and high strength and of nanofibers like moldability, light weight and flexibility.

In this study, the cellulose crystalline regions and amorphous susceptibility to hydrolysis of enzymes was used to produce cellulose nanocrystals (CNCs). Also, the synthesis ZnO-Ag nanoparticals by using the resulted CNCs as a new stabilizer was done, which led to the formation of the CNCs-ZnO-Ag composites. On the other hand, the characterization and antimicrobial activity of the composite was done.

Materials and Methods

1. Bacterial Cellulose (BC) production

Bacterial cellulose membrane was produced by *Gluconacetobacter xylinus NRRL B-43* using 250 ml conical flask of Sorbitol broth medium containing (g/l): peptone, 10.0; yeast extract, 10.0 and D-Sorbitol, 50; the pH was adjusted to 6.2 under static condition at 28°C for 7 days [15].

2. Cellulase production

2.1. Fungal isolation

The fungal isolated from a local soil sample, was grown on potato dextrose agar (PDA) plates and incubated at 30°C for 7 days. Purification of the fungus was done using the hyphal tip technique. The strain was then transferred into slants of PDA and kept at 4°C for further studies. A pure culture of the fungal isolate was identified at the National Research Centre, Cairo, Egypt.

2.2. Fungal growth enhancement medium

The medium used for preparation of activated fungal inoculums was containing (g/l): Glucose, 10.0; peptone, 5.0; yeast extract, 1.0; $MgSO_4$, 0.5 and KH_2PO_4, 1.0.

2.3. Fungal production medium

The medium use for production of carboxy methyl cellulase (CM-cellulase) was containing (g/l):- Beet pulp residue, 60; KH_2PO_4, 0.5; $MgSO_4$, 0.5; $NaNO_3$, 2.5 and 1000 ml distilled H_2O.

2.4. Assay of CM-cellulase activity

CM-cellulase was measured viscometrically[16,17].

3. Preparation of cellulose nanocrystal (CNCs):-

Bacterial cellulose was produced by *Gluconacetobacter xylinus NRRL B-43*. The cellulose was purified by boiling the pellicles in 0.2M aqueous NaOH solution for 30 min followed by several rounds of rinsing with distilled water until a neutral pH was attained in the drained water. The neat pellicles were mechanically disintegrated to a cellulosic paste using a laboratory blender at 5000–6000 rpm for about 10 min at room temperature. The cellulose paste was filtered through Whitman No. 1 filter paper to remove excess water prior to enzyme hydrolysis.

The enzyme used in the study has a specific enzyme activity of around 700 U/g. The paste (25 gm, wet weight) was suspended in 250 ml of acetate buffer (0.1 M, pH 5.0) to which 1 ml of cellulase was added with gentle stirring. This suspension was kept at 40°C for 24 hours in an incubator and the sampling was taken after 24 hours. Finally the mixture was centrifuged at 10,000 rpm (at 4°C for 20 min) to terminate the enzymatic reaction and to collect the cellulosic fragments. The cellulose sediment collected was further washed with distilled water several times to remove the residual enzyme present along with cellulose then kept in refrigerator at 4°C for further use[18].

4. Production of CNCs- ZnO-Ag composite

The Cellulose NanoCrystals-ZnO-Ag was synthesized as follows: ZnSO4 solution samples (50.0 mL, 10.0 wt %) were dispersed to CNCs suspensions (100.0 mL, 2.0 wt %) by magnetic stirring. After complete mixing a sodium hydroxide solution (5.0 mol/L) was added drop wise to the mixed solutions under continuous stirring at 80°C until pH > 10 was reached. After observing a milky color suspension, we added aqueous

$AgNO_3$ solutions (20 ml, 10.0 wt %) and the reaction was continued for 2 hours under strongly stirring. The products were collected through centrifugation and careful washing three times with distilled water. The final products were obtained by drying at 100°C for 1 hour for complete transformation of the remaining zinc hydroxide to zinc oxide [19].

5. Microbial sensitivity

5.1. Test microorganisms

The antimicrobial activity was done using various pathogenic microorganisms such as *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* as models for Gram-negative bacteria; *Staphylococcus aureus ATCC 43300S, ATCC 29213* and *Bacillus cereus* as models for Gram-positive bacteria and *Candida albicans ATCC 10231* as models for unicellular fungi.

5.2. Antimicrobial activity media

The media used for the antimicrobial activity of the (CNCs-ZnO-Ag) composite under study have the following compositions (g/l):- Nutrient agar medium: - D-glucose, 5.0; peptone, 5.0; meat extract, 5.0; NaCl, 5.0 and agar, 20.0; the pH was adjusted to 7. Used for growth of bacterial strains. Sabouroud agar medium: - dextrose, 40.0; peptone, 10.0 and agar, 20.0; the pH was adjusted to 7. Used for growth of unicellular fungi**.**

5.3. Antimicrobial potential Assay

The antimicrobial activity was evaluated by the disc diffusion method by using different models Gram-negative bacteria; Gram-positive bacteria and unicellular fungi. Sample powder was formed into disc shapes of 7 mm diameter and UV sterilized for 2 hours, then placed over the agar surface plates freshly inoculated with the test microorganisms (Nutrient agar medium for bacterial strains and Sabouroud agar medium for unicellular fungi). The petri-dishes were kept in a refrigerator for one hour to permit homogenous diffusion of the antimicrobial agent before growth of the test microorganisms and then plates were incubated at 37°C for 24 hours for Gram positive and Gram negative bacteria and at 28°C for 72 hours for unicellular fungi. The appearance of a clear inhibition zone around the sample in the inoculated petri-dishes is an indication of the antimicrobial activity [20].

6. Transmission Electron microscopy (TEM) of CNCs-ZnO-Ag composite:

The Cellulose NanoCrystals-ZnO-Ag composite was evaluated for their (CNCs-ZnO-Ag) size and shape characteristics by transmission electron microscopy. The TEM image was carried out using: Electron probe micro-analyzer JEOL – JXA 840A, Model Japan. Thin films of the sample were prepared on a coated copper grid by just placing a very small amount of the sample on the grid. Then the film on the TEM grid was allowed to dry and the images of nanoparticles were taken.

Results and Discussion

1. Fungal identification

The fungal isolated from a local soil sample, was transferred into slants of PDA and kept at 4°C. A pure culture of the fungal isolate was identified, at the National Research Centre of Egypt, as *Aspergillus niger* according to some cultural properties as well as certain morphological and microscopically characteristics.

2. Synthesis of cellulose nanocrystals (CNCs)

Depending on many parameters carboxymethyal cellulase enzyme can hydrolyze cellulose into oligosaccharides, glucose, small fragment or cellobiose. Although, it start with random cleavage in the long cellulose chain but at the end give many fractions of hydrolysis products. The cellulose crystalline regions are extra resistant to enzymatic hydrolysis because of the existing hydrogen bond between them, in contrast to the less consolidated amorphous zones with fewer hydrogen bonding [21]. The enzyme ability to hydrolyze the selective amorphous regions can be utilized in the elaboration of CNCs with likable properties. The process of enzymatic reaction is promoted because it is ecofriendly and cleaner than traditional acid hydrolysis procedures.

On the other hand, the native cellulose properties can be kept even in nanocrystal form. In this work, carboxymethyl cellulase enzyme from *Aspergillus niger* was used for preparation of bacterial cellulose nanocrystales (CNCs) at 1 ml concentration in acetate buffer of pH 5.0 at 40°C for 24 hours and this was the maximum conditions for the activity of enzymes[22].

3. Synthesis of cellulose nanocrystals-ZnO-Ag composites:-

The ZnO-Ag nanoparticals were prepared with new stabilizing agent cellulose nanocrystals to block the aggregation of the nanoparticals and promote its stability. The addition of zinc sulphate and $AgNO_3$ is serious for the formation of Ag^+ nanoparticals on the surface of ZnO[23]. Dispersability of CNCs in water is good and the suspension does not precipitate due to the presence of sulfate groups on the CNCs surface which introduce during the sulfuric acid hydrolysis and the plentiful of hydroxyl groups[24]. First, the Zn^{2+} cations absorbed onto The (OH^-) functional groups by electrostatic interaction between metallic cations and oxygen atoms of the polar hydroxyls. These process control the size of metallic particles by prevent its agglomeration. Second, the $Zn(OH)_2$ is slowly formed in CNCs by the addition of drop wise of NaOH solutions and under thermal conditions ZnO is formed. Also, Ag^+ ions are reduced to nanoparticales by additions of $AgNO_3$ in alkaline suspension.

4. Microbial sensitivity of CNCs-ZnO-Ag composite

The disc diffusion experiment was carried out against *Escherichia coli* ATCC25922and *Pseudomonas aeruginosa* as models for Gram-negative bacteria; *Staphylococcus aureus* ATCC 43300S, ATCC 29213 and *Bacillus cereus* as models for Gram-positive bacteria, and *Candida albicans* ATCC10231as models for unicellular fungi. As illustrated in Table (1) inhibition zone ranging from (16 - 20 mm) diameter for CNCs-ZnO-Ag composite were observed and this is only due to silver and zinc nanoparticles impregnated inside CNCs. It is worth mentioning the BC only lacing to antimicrobial activity but, these results clearly indicate that the antimicrobial activity is only due to the silver and zinc nanoparticles which were impregnated inside BC and not due to the microcrystalline cellulose. Furthermore, doped Ag reduces the ionization energy of acceptors in ZnO and consequently enhances the emission. Therefore, Ag ions can enhance the antimicrobial ability of ZnO [12].

Table (1): Microbial sensitivity of CNCs- ZnO- Ag composites against pathogenic tested microorganisms.

Tested pathogen organisms		Inhibition zone (mm) CNCs-ZnO -Ag composite
Grams negative	*Escherichia coli* ATCC25922	18.0
	Pseudomonas aeruginosa	16.0
Grams positive	*Staphylococcus aureus ATCC 29213*	20.0
	Staphylococcus aureus ATCC 43300S	17.0
	Bacillus cereus	16.0
Unicellular Fungi	*Candida albicans ATCC 10231*	19.0

5. TEM description of CNCs -ZnO-Ag composite

Transmission Electron Microscopy image of CNCs-ZnO-Ag composite at Figure (1) shows average size of 6-50 nm. About shape, the optic rounded and oval nanoparticles of CNCs-ZnO-Ag composite were detected, also, separated and conjugated nanoparticles were showed. It is worth mentioning that no separately silver or zinc particles were showed outside the cellulose, indicating the strong interaction of CNCs-ZnO-Ag composite.

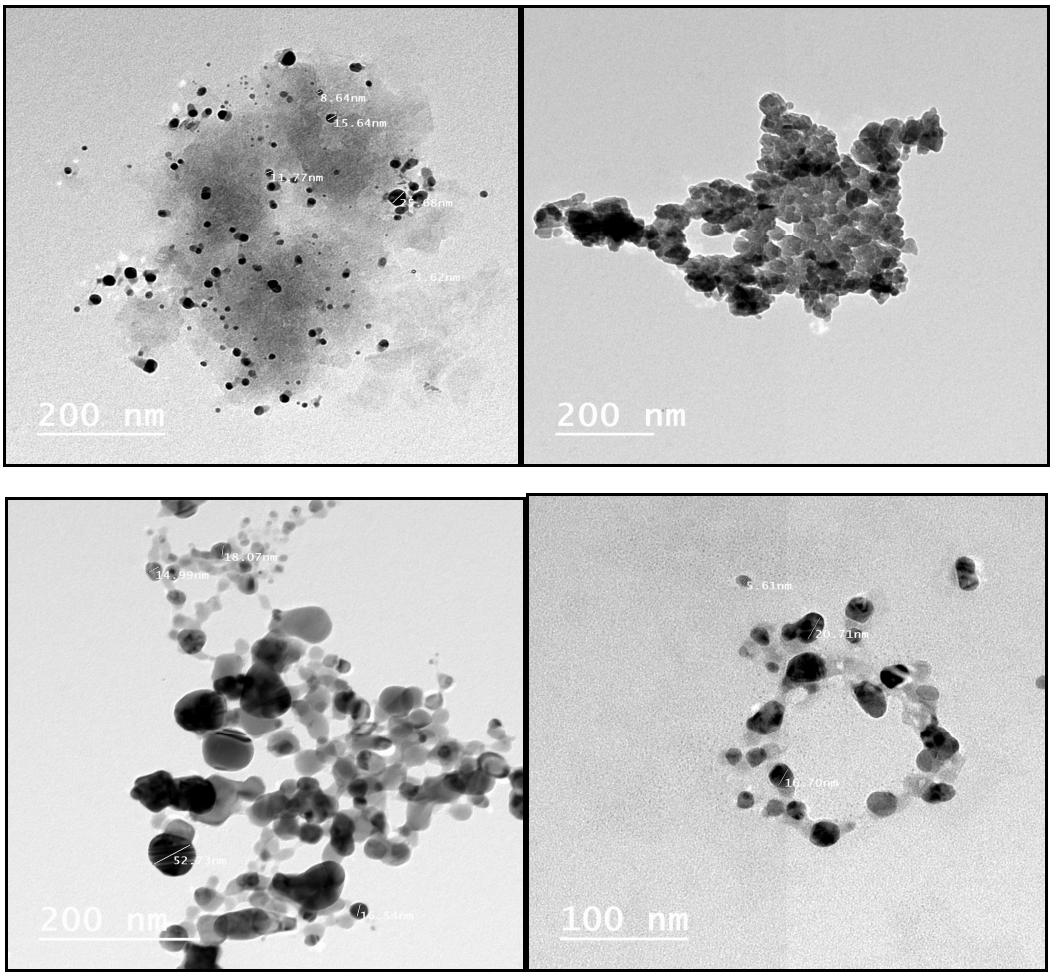

Figure (1): TEM description of CNCs -ZnO-Ag composite at magnification 100 and 200 nm.

References

1. Wang, L.; Sun,Y.; Wang, J.; Yu, A.; Zhang, H.; Song, D. Water-soluble ZnO-Au nanocomposite based probe for enhanced protein detection in a SPR biosensor system. J. Colloid Interface Sci. 2010, *351*, 392–397.
2. Zou, C.W.; Rao, Y.F.; Alyamani, A.; Chu, W.; Chen, M.J.; Patterson, D.A.; Emanuelsson, E.A.; Gao, W. Heterogeneous lollipop-like V2O5/ZnO array: A promising composite nanostructure for visible light photocatalysis. Langmuir 2010, 26, 11615–11620.
3. Yuan, J.; Choo, E.S.; Tang, X.; Sheng, Y.; Ding, J.; Xue, J. Synthesis of ZnO-Pt nanoflowers and their photocatalytic applications. Nanotechnolgy 2010, 21, 185606–185706.
4. Sathish Kumar, P.S.; Manivel, A.; Anandan, S. Synthesis of Ag-ZnO nanoparticles for enhanced photocatalytic degradation of acid red 88 in aqueous environment. Water Sci. Technol. 2009, 59, 1423–1430.
5. Sadaf, J.R.; Israr, M.Q.; Kishwar, S.; Nur, O.; Willander, M. White electroluminescence using ZnO nanotubes/GaN heterostructure light-emitting diode. Nanoscale Res. Lett. 2010, *5*, 957–960.
6. Yin, Z.Y.; Sun, S.; Salim, T.; Wu, S.X.; Huang, X.; He, Q.Y.; Lam, Y.M.; Zhang, H. Organic photovoltaic devices using highly flexible reduced graphene oxide films as transparent electrodes. ACS Nano. 2010, *4*, 5263–5268.
7. Dong, H.; Strawhecker, K.E.; Snyder, J.F.; Orlicki, J.A.; Reiner, R.S.; Rudie, A.W.; Cellulose nanocrystals as a reinforcing material for electrospun poly(methylmethacrylate) fibers: Formation, properties and nanomechanical characterization. *Carbohydr. Polym.* **2012**, *87*, 2488–2495.
8. Yin, Y.T.; Que, W.X.; Kam, C.H. ZnO nanorods on ZnO seed layer derived by sol–gel process. J. Sol-Gel Sci. Technol. 2010, *53*, 605–612.

9. Karunakaran, V.; Rajeswari, P.; Sankar, G. Antibacterial and photocatalytic activities of sonochemically prepared ZnO and Ag-ZnO. J. Alloys Compd. 2010, 508, 587–591.
10. Chen, R.Q.; Zou, C.W.; Bian, J.M.; Sandhu, A.; Gao, W. Microstructure and optical properties of Ag-doped ZnO nanostructures prepared by a wet oxidation doping process. *Nanotechnology* **2011**, *22*, 105706–105713.
11. Liu, Y.; Kim, H. Characterization and antibacterial properties of genipin-crosslinked chitosan/poly (ethylene glycol)/ZnO/Ag nanocomposites. *Carbohydr. Polym.* **2012**, *89*, 111–116.
12. Karunakaran, C.; Rajeswari, V.; Gomathisankar, P. Optical, electrical, photocatalytic, and bactericidal properties of micro wave synthesized nanocrystalline Ag-ZnO and ZnO. *Solid State Sci.* **2011**, *13*, 923–928.
13. Liu, R.L.; Huang, Y.X.; Xiao, A.H.; Liu, H.Q. Preparation and photocatalytic property of mesoporous ZnO/SnO_2 composite nanofibers. J. Alloys Compd. 2010, 503, 103–110.
14. Patel, A.C.; Li, S.X.; Wang, C.; Zhang, W.J.; Wei, Y. Electrospinning of porous silica nanofibers containing silver nanoparticles for catalytic applications. Chem. M+ater. 2007, *19*, 1231–1238.
15. El-Waseif A. A. and El-Ghwas E. D.; Involving the Silver Particles into Microbial Membrane to Improve the Biological Activity and Characterization. International Journal of PharmTech Research 2016: 9(5) 16-22.
16. Child JJ, Eveleigh DE, Sieben AS ; Determination of cellulose activity using hydroxyethylcellulose as substrate. Canadian Journal of Biochemistry 1973;51: 39–43.
17. Eriksson KE, Hollmark BH; Kinetic studies of the action of cellulose on sodium carboxymethyl cellulose. Archives of Biochemistry & Biophysics 1969; 133: 233–237.
18. George J., K.V. Ramana, S.N. Sabapathy, J.H. Jagannath, A.S. Bawa, Int. J. Biol. Macromol. 2005; 37: 189–194.
19. Azizi S., Mansor B. Ahmad, M. Z. Hussein and N. A. Ibrahim. Synthesis, Antibacterial and Thermal Studies of Cellulose Nanocrystal Stabilized ZnO-Ag Heterostructure Nanoparticles. Molecules 2013; 18: 6269-6280.
20. Y. Nishiyama, P. Langan, H. Chanzy, J. Am. Chem. Soc. 2002; 124: 9074–9082.
21. S. Ahola, X. Turon, M. Osterberg, J. Laine, O.J. Rojas, Langmuir 2008: 24: 11592–11599.
22. Dong, X.M.; Kimura, T.; Revol, J.-F.; Gray, D.G. Effects of ionic strength on the isotropic-chiral nematic phase transition of suspensions of cellulose crystallites. Langmuir 1996, 12, 2076–2082
23. Tian, C.; KaiPan, W.; Zhang, Q.; Tian, G.; Zhou, W.; Fu, H. One pot synthesis of Ag nanoparticle modified ZnO microspheres in ethylene glycol medium and their enhanced photocatalytic performance. *J. Solid State Chem.* 2010, *183*, 2720–2725.
24. Oluwafemi, F. and Debiri, F.: Antimicrobial effect of Phyllanthus amarus and Parquetina nigrescens on Salmonella typhi. Afri. J. Biom. Res. 2008: 11: 215-219.

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