



Synthesis of copper nanoparticles and their antimicrobial performances in natural fibres

M.N.K. Chowdhury, M.D.H. Beg, Maksudur R. Khan*, M.F. Mina

Faculty of Chemical & Natural Resources Engineering, Universiti Malaysia Pahang, 26300 Gambang, Kuantan, Pahang, Malaysia

ARTICLE INFO

Article history:

Received 21 November 2012

Accepted 9 February 2013

Available online 17 February 2013

Keywords:

Nanoparticles

FTIR

Antimicrobial activity

Natural fibre

ABSTRACT

Copper nanoparticles (CuNPs) were synthesized at ambient atmospheric condition by the chemical reduction method. The reduction of the solution of copper chloride salt in the polyvinyl alcohol system was done by sodium borohydride solution. The ultraviolet–visible spectroscopy, Fourier transformed infrared spectroscopy, transmission electron microscopy and X-ray diffraction studies were employed to analyse the formations and sizes of nanoparticles. The average size of CuNPs was measured to be 3 nm. The synthesized CuNPs were impregnated in natural fibres and their antimicrobial performances were assessed against both the Gram-positive and Gram-negative bacteria, showing more antibacterial activity in *Escherichia coli* than *Staphylococcus aureus* along with ~7% antifungal activity.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Synthesis of novel nano material is a significant focal point of numerous research spheres, where continuous efforts are being made by scientists and engineers for both industrial and technological advancements. In this context, nanoparticles (NPs) have received extensive interests because of their outstanding physical, chemical, electronic, magnetic, catalytic and surface properties in manifold applications [1,2]. Among metals, copper NPs (CuNPs) have drawn much attention owing to their versatile applications for instance, catalytic, electrical, optical, and antifungal/antibacterial purposes. To synthesize CuNPs, notable commonly used methods are chemical reduction, co-precipitation, sol–gel processing, template synthesis, thermal reduction, microwave irradiation methods, vacuum vapour deposition and laser ablation. Of these, the chemical reduction process (CRP) has emerged as an enthusiastic technique to control the size of nanoparticles [3]. However, a novel formulation under CRP at ambient atmosphere has to be necessarily employed for developing CuNPs with a size of a few nanometres (1–10 nm). On the other hand, Cu is known to exhibit a broad-spectrum biocide and effectively inhibit the growth of bacteria, fungi and algae [4]. Recent studies revealed that CuNPs showed a good antibacterial activity [5], and these nanoparticles coated surfaces suitably served medical requirements. Hence, the present work has been undertaken to synthesize CuNPs, which, if embedded in cellulose containing materials, is expected to improve their antimicrobial activity so that metallic

NPs reinforced materials can be exploited in due applications. Antibacterial performance of CuNPs impregnated oil palm empty fruit bunch (EFB) fibres has been studied by using *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) bacteria. The antifungal activity of CuNPs impregnated fibres has also been studied.

2. Experimental

Raw materials were copper (II) chloride salt ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$), sodium hydroxide, polyvinyl alcohol (PVA), sodium borohydride (NaBH_4) Reagent Plus, ascorbic acid, 60% solution of 3-chloro-2-hydroxypropyltrimethyl ammonium chloride (CHPTAC) and untreated EFB (UEFB) fibres. The types of bacteria were ATCC. The synthesis of copper nanoparticles was done with 0.5% PVA solution in deionized (DI) water. Then, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (250 mg) and ascorbic acid (5 g) were subsequently added in 100 mL PVA solution with vigorous stirring. About 7.0 mL solution of sodium borohydride (0.347 M) was injected drop-wise to the reaction mixture with continuous stirring for 2 h. UEFB fibres were cationized by 36% CHPTAC solution by maintaining a liquor ratio of 1:30 in a water bath. The cationized EFB (CAEFB) fibres were removed from the bath, rinsed several times with water, neutralized and then dried at the ambient temperatures. The CAEFB fibres were introduced in the synthesized nanocopper sol (214 mg/L), maintaining the fibre to sol ratio of 1:30 with continuous shaking by a mechanical shaker for 12 h. Then, the CuNPs loaded CAEFB (CuNP-CAEFB) fibres were removed, rinsed with water, and dried in the dark place at room temperature.

Ultraviolet–visible (UV–vis) absorption spectroscopy was performed by a Perkin-Elmer Lambda 950 UV–visible double

* Corresponding author. Tel.: +60 9 549 2872.

E-mail address: mrkhancep@yahoo.com (M.R. Khan).

beam spectrophotometer. Fourier-transformed infrared (FTIR) spectra of sols were taken with a PerkinElmer Spectrum 100 FTIR Spectrometer over the frequency range $4000\text{--}650\text{ cm}^{-1}$. X-ray diffraction (XRD) data were collected by using a Rigaku MiniFlex II, Japan, within scattering angle (2θ) between 10 and 50° , using the wavelength $\lambda = 1.541\text{ \AA}$. The average size of the crystallites, D_{hkl} , was determined with the full width at half-maximum peak by using the following Scherer formula [6]. Transmission electron microscopy (TEM) was performed by a LEO 912 AB EFTEM operating at 120 kV . Atomic absorption spectrophotometry (AAS) was carried out by a Perkin Elmer Analyst 400 apparatus to measure the amount of impregnated copper nanoparticles in EFB fibres. Fibres were investigated by field emission scanning electron microscope (FESEM) of JEOL JSM-7600F, USA. The antibacterial activity of the CuNP-CAEFB fibre was investigated against *S. aureus* and *E. coli*. The suspension of bacteria cells was used in phosphate buffered saline (PBS) solution where the final density was 10^6 cells/mL. Initially, UEFB and CuNP-CAEFB fibres (1 cm -length) were washed by 75% ethanol to kill bacteria, and then immersed in the bacterial suspension (5 mL) in an Erlenmeyer flask, wherein the solution was shaken at 200 rpm at 37°C . The viable cell counts of bacteria were measured by surface spread plate method. After $0, 0.5, 1,$ and 2 h , 0.2 mL of bacterial culture was used from the Erlenmeyer flask and then serially diluted with PBS, and the steps were repeated. Onto the solid growth agar plates, 0.1 mL diluent of the sample was spread. After incubation of the plates at 37°C for 24 h , the number of viable cells was counted and multiplied by the dilution factor. For testing the antifungal activity as per AATCC test method 30-2004, UEFB and CuNP-CAEFB fibres were buried under soil. After one week of exposition, the samples were then subjected to mechanical test. The tensile properties of UEFB and CuNP-CAEFB fibres before and after burials were measured using a Shemadzu Universal Tensile Testing Machine (Japan). The single fibre was cut to a length of about 5 cm , and its tensile strengths (TS) were measured using a 5 kN load. The average values were evaluated from the data of 25 individual fibres.

3. Results and discussion

A change of sol colour is observed after the addition of reducing agent, where the light-blue coloured aqueous solution changes to wine red, which is a convincing indication of the formation of CuNPs in the sols. The UV-vis spectra of copper sols synthesized (a) at initial stage and (b) after two weeks are shown in Fig. 1(i). A sharp edge observed at $\sim 516\text{ nm}$ for both spectra represents the existence of metallic CuNPs. It was reported that when the particle size is $\sim 5\text{ nm}$ or larger, the spectrum shows the surface plasmon resonance (SPR) peak, which becomes prominent when the particles grow much larger in size [7]. Since no SPR peak is visible in our observed spectrum, the size of synthesized CuNPs is expected to be $< 5\text{ nm}$. The colour of nanocopper sol has been found to sustain at least for two weeks. This stability of sol colour may be attributed to the presence of anions, namely borohydride and ascorbic acid. The sol remains stable in contact with air, suggesting that the synthesized copper sol is resistant to oxidation. TEM image clarifies that the particles developed in the sol are in sizes in the order of a few nanometres with spherical shape. Analysis from 100 to 500 particles of diameters $1.2\text{--}10.5\text{ nm}$ shows the average size of $3.5 \pm 1.1\text{ nm}$.

Fig. 1(ii) (a) illustrates the FTIR spectra of solution before and after nanoparticle formation. A broad absorption band of hydroxyl (O–H) groups of PVA chains appear at 3359 cm^{-1} and 3341 cm^{-1} before and after NPs formation, respectively, showing 18 units red shift of this polar group. This shifting of wave number demonstrates that PVA

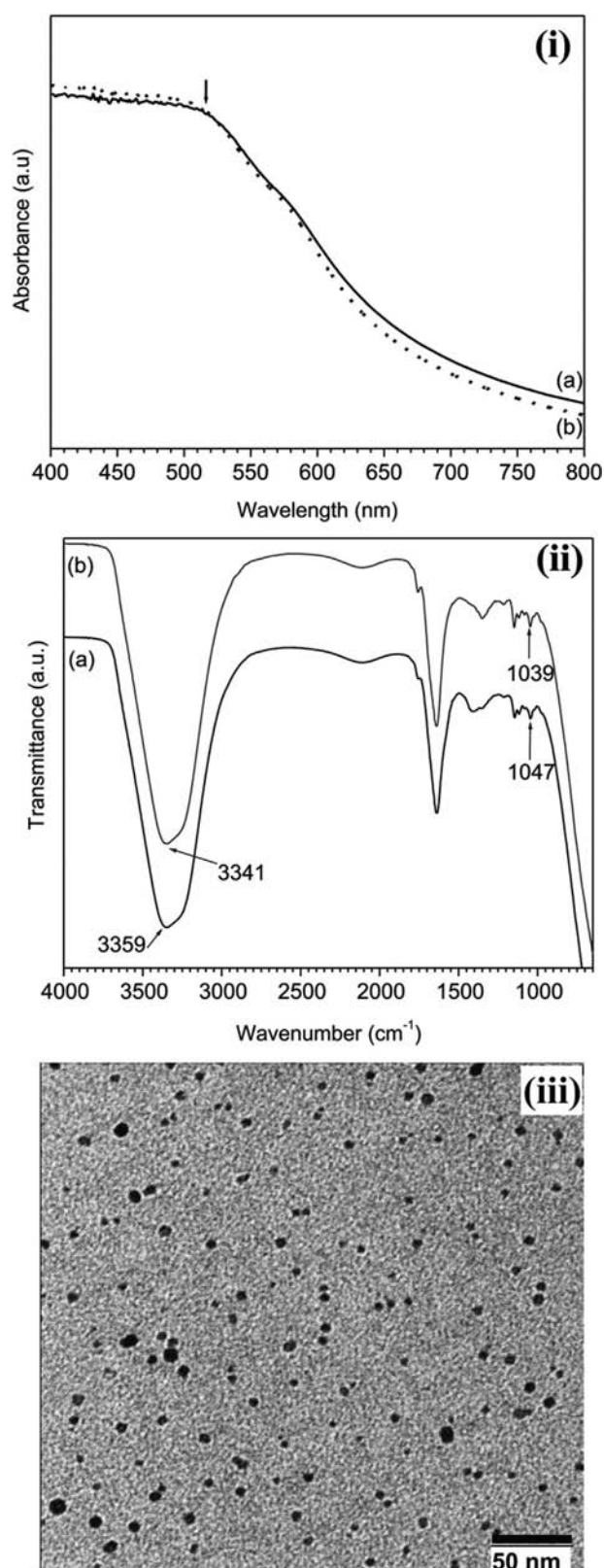


Fig. 1. (i) The UV-vis spectra of nanocopper sols: (a) at initial stage and (b) after two weeks of synthesis. (ii) The FTIR spectra of nanocopper sols: (a) before and (b) after NP formation. (iii) A TEM image of CuNPs.

molecules facilitate coordination with Cu^{2+} species. Such coordination through the ester bond of PVA to Cu species due to the electrostatic attractions was described elsewhere [8]. The ester bond is located at 1047 cm^{-1} before NP formation and shifted to

1039 cm^{-1} after NP formation, indicating the coordination of polar O–H groups with Cu species. The morphologies and sizes of CuNPs in the sol were monitored by TEM as shown in Fig. 1(iii). The morphologies and sizes of CuNPs were also observed by FESEM as shown in Fig. 2(i), which clearly demonstrated the bonding between NPs and fibre. The amount of copper adsorptions on CAEFB fibres from sols measured by AAS is found to be $2590\text{ }\mu\text{g/g}$. When CAEFB fibres are immersed in a nanocopper sol, CuNPs stabilized by

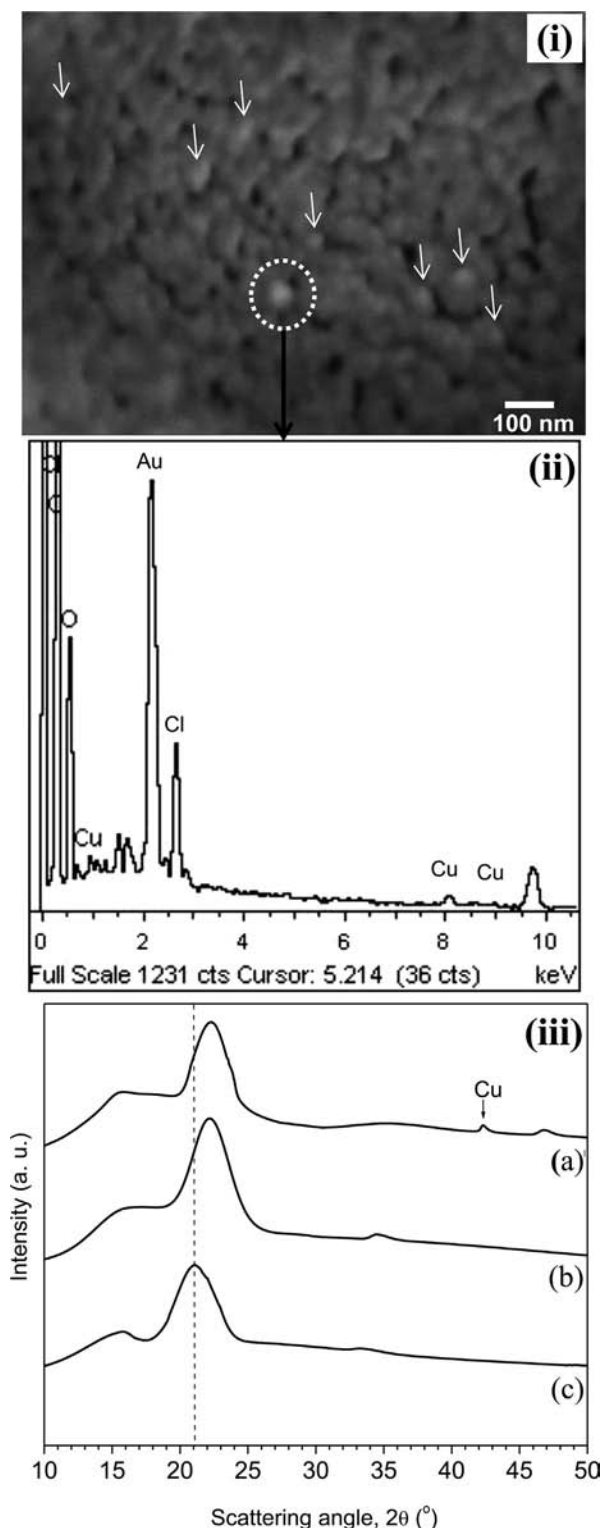


Fig. 2. (i) FESEM image of CuNP-CAEFB fibres. (ii) EDX spectrum of marked zone of (i). (iii) XRD patterns of (a) UEFB, (b) CAEFB, and (c) CuNP-CAEFB fibres.

negatively charged PVA spheres can readily be adsorbed by the positive sites of cationized fibres. A large amount of positive charges may cause the charge reduction at the fibre surface because of cationization of EFB fibres, thereby increasing the adsorption capability of functionalized copper nanoparticles due to an attractive interaction between fibres and CuNPs. The X-ray diffractograms of different fibres are illustrated in Fig. 2(iii). The peak appearing at $2\theta=42.3^\circ$ for CuNP-CAEFB fibre has been reportedly attributed to the 111 reflection from the cubic copper crystal [9]. This finding demonstrates the CuNPs impregnation in the fibres. The average size of CuNPs as analysed from (1 1 1) peak is $\sim 17\text{ nm}$.

Table 1 depicts the TS of different fibres before burial (BB) and after burial (AB) under soil. Analyses show that the increase in TS of CuNP-CAEFB fibre from UEFB fibre before burial is 25%. This increase in TS is rational, because the functionalized CuNPs can enter into fibre matrices and act as filler or plasticizer, which contribute to load sharing during stretching the fibres. On the other hand, the TS of UEFB and CuNPs-CAEFB fibres after soil burial decreases by 32% and 25% from their respective TS before burial, resulting in $\sim 7\%$ antifungal activity. However, the TS-decrease of UEFB fibres is more than CuNP-CAEFB fibres, demonstrating that CuNPs reinforced fibres are more durable than the control ones. This result may be explained by the facts that the metallic nanoparticles can show a certain degree of sterilization, because the catalytic properties of metallic species partly help to create active oxygen in water, which dissolve the organic substances to maintain sterilizing effect [5,10]. To evaluate the antibacterial activity of the synthesized CuNPs, *S. aureus* and *E. coli* were chosen. The results presented in Table 1 shows the distinct differences between two types of fibres for antibacterial activity. Apparently, UEFB fibres had no antibacterial activity as the number of viable bacteria remains static like the initial stage. Conversely, viable counts of 4-log reduction are noticeable on CuNP-CAEFB fibre within 0.5 h exposure for *E. coli* and *S. aureus*, respectively. Moreover, both these bacteria were annihilated by CuNP-CAEFB fibre after 1 h of exposure. There is an interaction between bacteria and fibre at the surface of CuNP-CAEFB. Since Cu is one of the long-lasting biocide and the release of Cu is very slow with small quantities [11], the CuNP-CAEFB fibre shows a prompt sterilization to both *E. coli* and *S. aureus*, which might be due to the discharge of Cu ions into culturing medium. The observed findings suggest that the synthesized CuNPs can be considered as potential biocide in composite materials.

Table 1
Antimicrobial performances of synthesized CuNPs impregnated in EFB fibres.

Antifungal test								
Test	Tensile strength (TS), MPa							
Fibres	UEFB				CuNP-CAEFB			
	Before burial	After burial	Before burial	After burial	Before burial	After burial	Before burial	After burial
Values	259 ± 8.9	176 ± 7.8	347 ± 9.2	261 ± 9.3				
Antibacterial test								
Fibres	Log (Viable cell numbers/mL)							
	<i>Escherichia coli</i>				<i>Staphylococcus aureus</i>			
	0 h	0.5 h	1 h	2 h	0 h	0.5 h	1 h	2 h
Control (no fibre)	6	6	6	6	6	6	6	6
UEFB	6	6	6	6	6	6	6	6
CuNP-CAEFB	6	2	–	–	6	3	–	–

4. Conclusions

CuNPs are synthesized in the aqueous phase by means of sodium borohydride, where they are functionalized with PVA. TEM analysis confirms that the NP has an average size of 3.5 nm and is spherical in shape. UV–vis spectroscopy confirms the stability of the sols for at least two weeks and indicates the formation of CuNPs of size < 5 nm. The observed mechanical and antimicrobial properties of the synthesized CuNPs impregnated EFB fibres make them potentially ideal reinforcing agents to enhance their mechanical property and durability as well as the synthesized CuNPs can effectively be used against the microbes.

References

- [1] Peng X, Manna L, Yang W, Wickham J, Scher E, Kadavanich A, et al. Shape control of CdSe nanocrystals. *Nature* 2000;404(6773):59–61.
- [2] Alivisatos AP. Semiconductor clusters, nanocrystals, and quantum dots. *Science* 1996;271(5251):933–7.
- [3] Yang M, Zhu JJ. Spherical hollow assembly composed of Cu₂O nanoparticles. *J Crystal Growth* 2003;256:134–8.
- [4] Dollwet HHA, Sorenson JRJ. History uses of copper compounds in Medicine. *J Trace Element Med* 1985;2(2):80–7.
- [5] Cady NC, Behnke JL, Strickland AD. Copper-based nanostructured coatings on natural cellulose: nanocomposites exhibiting rapid and efficient inhibition of a multi-drug resistant wound pathogen, *a. baumannii*, and mammalian cell biocompatibility in vitro. *Adv Funct Mater* 2011;21(13):2506–14.
- [6] Inagaki T, Siesler HW, Mitsui K, Tsuchikawa S. Difference of the crystal structure of cellulose in wood after hydrothermal and aging degradation: a NIR spectroscopy and XRD study. *Biomacromolecules* 2010;11(9):2300–5.
- [7] Nadagouda MN, Varma RS. A greener synthesis of core (Fe, Cu)-shell (Au, Pt, Pd, Ag) nanocrystals using aqueous vitamin C. *Crystal Growth Des* 2007;7(12):2582–7.
- [8] Zhang HX, Siegert U, Liu R, Cai WB. Facile fabrication of ultrafine copper nanoparticles in organic solvent. *Nanoscale Res Lett* 2009;4(7):705–8.
- [9] Xie WYH, Chen L, Li Y, Zhang C. Synthesis and characterization of monodispersed copper colloids in polar solvents. *Nanoscale Res Lett* 2009;4(5):465–70.
- [10] Chattopadhyay DP, Patel BH. Improvement of physical and dyeing properties of natural fibres through pre-treatment with silver nanoparticles. *Indian J Fibre Text* 2009;34(4):368–73.
- [11] Drelich J, Li B, Bowen P, Hwang JY, Mill O, Hoffman D. Vermiculite decorated with copper nanoparticles: novel antibacterial hybrid material. *Appl Surf Sci* 2011;257(22):9435–43.