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Structural, Antimicrobial and Electrochemical Properties of Cu/TiO₂ Nanocomposites

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ABSTRACT

The antimicrobial properties of copper (Cu) based titanium dioxide (TiO₂) nanocomposites have been actively investigated in the present study. Sonochemical method was employed for the synthesis of nanocomposites having different wt.% ratios of Cu, TiO₂ nanomaterials. In Cu-based TiO₂ nanocomposites, the size of Cu nanoparticle plays a crucial role in deciding the bactericidal performance activity. The XRD (X-ray diffraction), FTIR (Fourier transform infrared spectroscopy), SEM (Scanning electron microscope), HR-TEM (High-resolution transmission electron microscope) and cyclic voltammeter used to analyze crystallite size, functional groups, morphology, particle size and electrochemical activity respectively of well-formed nanocomposites. Detailed studies on antimicrobial activity of prepared nanomaterials are carried out on Gram positive, Gram negative and Yeast i.e., *Staphylococcus aureus* (ATCC 25923); *Bacillus subtilis* (ATCC 6633); *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231) by disc diffusion method. The obtained antibacterial activity was correlated with electrochemical studies, originate to be best suitable for the present application. The crystallite size was calculated from XRD and is found to be 16.6 – 22.7 nm and 42.2 – 56 nm for Cu/TiO₂ at different wt.% ratio respectively. Zone of inhibition is increasing as the concentration of Cu based TiO₂ nanocomposites are increasing. The result showed that Cu based nanocomposites has a very promising property as an antimicrobial material and can be utilized in wide range of biomedical applications.

1. Introduction

Nanotechnology is a field of science with huge latent applications. Nanoparticles (NPs) are materials in the range of 100 nm or less. Nanoparticles, irrespective of their chemical constituents have distinctive and interesting chemical and physical properties from their bulk counterparts because of their size and high surface area to volume ratios. Metal nanoparticles are of high interest to research in the areas of Nano electronics, Nano biotechnology, catalysis etc. Metal oxide nanoparticles have a great potential in the field of Nano medicine especially in diagnosis & therapeutics due to antimicrobial activities and their selectivity towards toxic biological systems [1-4]. Among various metal nanoparticles, copper (Cu) nanoparticles have attracted more attention due to its magnetic, catalytic and antibacterial/antifungal properties [5-8]. Titanium dioxide (TiO₂) nanoparticles exhibits high catalytic activity and efficient antibacterial activity [9]. TiO₂ nanoparticles decompose organic compounds by the formation and constant release of superoxide ions, hydroxyl radicals when exposed to non-lethal ultraviolet light. TiO₂ nanoparticles (NPs) can reflect and scatter UVA and UVB radiations and have gained popularity as inorganic physical sunscreen these NPs induce cyto- and Geno toxicity through oxidative stress [10]. For copper nanoparticles synthesis, chemical reduction with reducing and stabilizing agents is the most appropriate procedure among all as it is simple and produces nanoparticles with controlled size and shape and avoids oxide formation and also it is most economical. For metal oxide nanoparticles synthesis (TiO₂), solution combustion method is suitable as it is modest approach to decrease the particle size. Sonochemical method is selected for synthesis of Cu based TiO₂ nanocomposites, because it is a very simple and fast which is performed under atmospheric pressures.

The present study, deals with synthesis of copper nanoparticles chemical reduction, TiO₂ nanoparticles were synthesized by solution combustion method. Present study is deeply concentrated on synthesis of Cu/TiO₂ nanocomposite by sonochemical method, characterizations by XRD, FTIR, HR-TEM, SEM/EDAS, cyclic voltammeter and antimicrobial

application on gram positive, gram negative bacteria and yeast species. The novelty of the current study is to know nanocomposites average crystallite size variation by different wt.% ratios, antimicrobial effect on nanocomposites by varying different wt.% ratio and correlation antibacterial with electrochemical studies.

2. Experimental Methods

2.1 Materials

Copper acetate Cu(CH₃COO)₂ (98% purity), polyvinylpyrrolidone (PVP) k-40 (98% purity), hydrazine hydrate (N₂H₄.H₂O) (97% purity), Tween-80 (99% purity), titanium isopropoxide Ti[OCH(CH₃)₂]₄ (97% purity), dextrose (C₆H₁₂O₆) (98% purity), Mueller Hinton Agar (MH agar) (97% purity) all are M/s by Sigma-Aldrich, antibiotic co-trimazole (Co) 25 µL (97% purity) was procured from a local chemist store. Gram positive, gram negative bacteria and yeast were taken from Centre for Environment, IST, JNTUH, and India. Distilled water was used throughout the chemical synthesis and antimicrobial procedure.

2.2 Synthesis of Cu and TiO₂ NPs

The Cu NPs were synthesized by Chemical reduction method. 2:1 ratio of Copper acetate to PVP k-40 are dissolved in distilled water and kept it on magnetic stirrer for continuous stirring about 10-15 min. To the above solution, hydrazine hydrate is added drop wise to reduce the material. Color changes of the solution were observed, initial blue to sea green, yellow, orange, brick red and finally reddish brown that signified the reaction has terminated and the temperature was maintained at 75 °C. The formation of copper ring around the beaker indicated the formation of copper nanoparticles. The reduced mixture was purified by ethanol and water through filtration. TiO₂ nanoparticles synthesis is as follows: Ti[OCH(CH₃)₂]₄ oxidizer is taken in required stoichiometric amounts in water. Dextrose fuel (0.01 M) was added to the oxidizer to form a clear solution, further non-ionic Tween 80 (0.01 M) was added to the prepared solution and stirred for 30 minutes and heated on a hot plate at 250 °C. The formed nanoparticles were calcined at 400 °C for 3 hours.

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2.3 Synthesis of Cu/TiO₂ Nanocomposites (NCs)

Cu and Cu/TiO₂ NCs were prepared by sonochemical method in different weight percentages of Cu and TiO₂ nanoparticles were taken (1:1, 1:2, 1:3, 2:1, 2:3, 3:1 and 3:2) and mixed using ethanol solvent in different beakers. Sonication process was carried out to form nanocomposite material with varying parameters like frequency of 20 kHz, Ultra sound power of 60 - 90 W, time for 30 minutes. Later, solution was centrifuged to 2000 rpm for 30 minutes. Then the material was dried at 100 °C in incubator. The resulted material thus formed is Cu nanocomposite, further these materials were characterized by various techniques and implemented on antibacterial application.

2.4 Characterization of Nanocomposites

Crystal structure and crystallite size of obtained nanocomposites were measured using XRD (Bruker D8 advance X-Ray Diffractometer) functioned with 40 kV & 30 mA using CuK α radiation ($\lambda=0.154$ nm) between 2 θ ° diffraction angles (20°- 80°). Morphology of the obtained nanocomposites was analyzed using electron microscopes FESEM, EDAS: ZEISS Scanning Electron Microscope & HR-TEM: JEM-100 CXII Transmission Electron Microscope. Surface functional groups were analysed by using Bruker FTIR (Fourier transform infrared spectroscopy) spectrophotometer in transmission mode. Particle size distribution was analyzed by Dynamic Light Scattering (DLS) principle SZ-100 equipped with a 532 nm laser. Antimicrobial activities of sonochemically processed nanocomposites were carried out on *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Candida albicans* using disc diffusion method. Mueller Hinton Agar medium is used to carry out the growth of the bacteria. A standard antibiotic (co-trimoxazole) test was accessed for Zone of Inhibition during the sensitivity.

2.4 Antibacterial Susceptibility Test

The antibacterial properties of as-prepared Cu/TiO₂ nanocomposites were performed by a qualitative method against microorganisms using disc diffusion method [11]. Gram-positive and gram-negative bacteria are cultured on Mueller–Hinton medium whereas yeast is cultured on potato dextrose medium. The bacteria *S. aureus* (ATCC 25923, gram-positive bacteria, 100 mg/ μ L); *B. subtilis* (ATCC 6633, Gram-positive bacteria, 100 mg/ μ L); *E. coli* (ATCC 25922, gram-negative bacteria, 100 mg/ μ L) and yeast *C. albicans* (ATCC 10231, yeast, 100 mg/ μ L) were inoculated into nutrient broth medium and incubated for 2 to 5 h at 37 °C until it reached turbidity of 0.5 Mc Farland standard. Liquid Mueller Hinton agar (10 mL) with pH 7.3 \pm 0.2 at 20 °C is poured in sterilized petri plates and allowed to solidify. The surfaces of the solidified agar plates are allowed to dry in the incubator before swabbing the microorganisms on the surface of agar plates. Microbial culture suspension (100 μ L) in broth having 97 colony/mL was swabbed onto the agar surface and allowed to dry. Cu/TiO₂ nanocomposites are suspended in sterilized DW and blank sterilized Whatman filter paper (6 mm diameter) are loaded with the suspension. Nanocomposites loaded discs were placed onto the surface of agar plates using sterile forceps. The experiments were carried out in duplicate & the diameters of inhibition zones measured after 24 hours of incubation at 37 °C. For monitoring any contamination during testing an additional media control plate and antibiotic (co-trimoxazole) were placed separately. Inhibition rates were calculated by using the following equation,

$$\text{Inhibition(\%)} = \frac{A_0 - A}{A_0} \times 100 \quad (1)$$

where A_0 = absorbance of the control (antibiotic) and A = test sample.

2.5 Electrochemical Studies

Electrochemical experiments performed by Metrohm AutoLab B.V (JNTUH, India). The three-electrode system consisting of working, reference and counter electrode as a modified glassy carbon electrode (GCE diameter of 3 mm), saturated Ag/AgCl and Pt wire in a electrolyte of 0.1 M NaOH which are purged with deoxygenated N₂ gas and used to study the electrochemical properties.

3. Results and Discussion

3.1 Structural Analysis of Cu/TiO₂ NCs

The XRDs pattern in Fig. 1, the pure nanocomposites obtained after sonochemical process showed intense peaks at the 2 θ angles with hkl planes are 43.3° (1 1 1) and 74.1° (2 2 0) respectively for Cu NPs. The patterns show good match with JCPDS card # 89-2838. According to the JCPDS card details, the obtained NCs exhibit a cubic system with a face centered cubic lattice structure.

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Crystallite sizes were calculated using Bragg's equation, $D = \lambda / \beta \cos \theta$, where β = FWHM (Full with half maxima), D = crystallite size, λ = wave length of the X-ray, θ = Bragg's angle. The synthesis was carried by varying the ratio of Cu:TiO₂ i.e. 1:1, 1:2, 1:3, 2:1, 2:3, 3:1 and 3:2, no extra peaks observed. Fig. 1 indicates the average crystallite size of composites. It clearly shows that the average crystallite of Cu/TiO₂ nanocomposites is increasing with TiO₂ concentration. Indeed, concentration is directly proportional to the crystallite size.

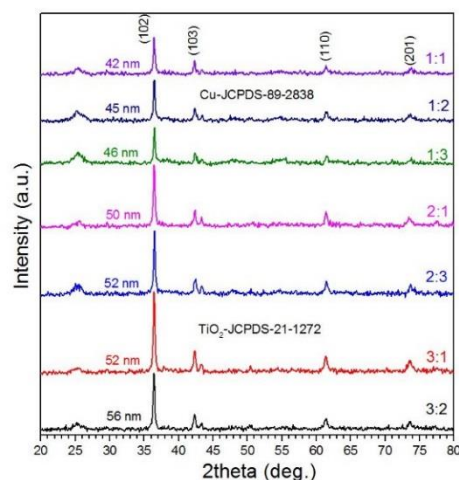


Fig. 1 XRD patterns and average crystallite size of Cu/TiO₂ NC at different wt.%

3.2 Morphological Analysis of Cu/TiO₂

3.2.1 FESEM – EDS Analysis

The morphology of obtained Cu/TiO₂ NC's from sonochemical method shown in Fig. 2a indicates semi porous nature of the sample. Fig. 2b indicates the presence of particles ranging from 60 – 150 nm. Fig. 2c EDS spectrum mainly shows three types of signal peaks corresponds to copper, titanium and oxygen where EDS table clearly shows the elemental composition. During the complete scan rate procedure no other element was detected. Due to high porosity, these nanocomposites are applicable for biomedical applications like antimicrobial activity, drug delivery, MRI etc. EDS report (Fig. 2c) confirms the chemical composition/ contaminants present in the synthesized sample.

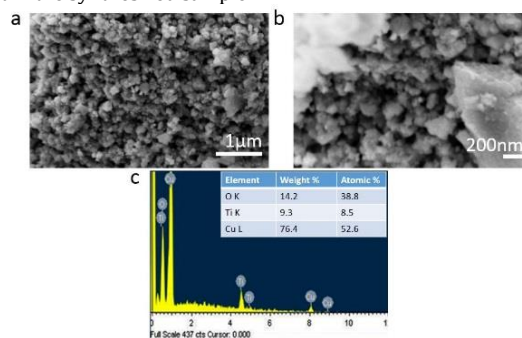


Fig. 2 (a, b) SEM images and (c) EDS spectrum of Cu/TiO₂ nanocomposites;

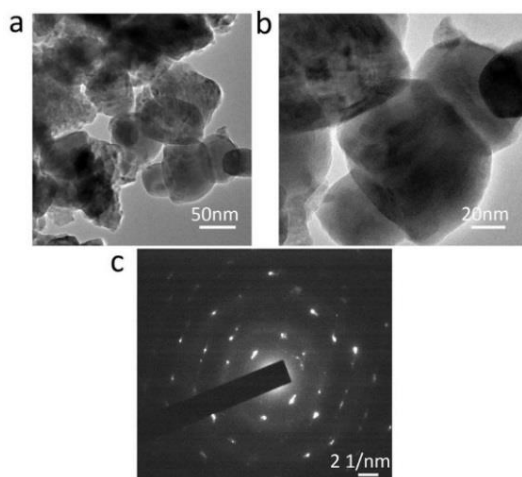


Fig. 3 (a, b) HRTEM image of Cu/TiO₂ NCs and (c) SAED pattern

3.2.2 HRTEM Analysis of Cu/TiO₂ NCs

The nanocomposites well dispersed in colloidal solutions are evident in HR-TEM micrograph. HR-TEM was carried out to analyze the shape and size of the Cu/TiO₂ NCs. Figs. 3a and b depict the average particle size of Cu/TiO₂ nanocomposites is 56 nm with the presence of tetragonal, cubic structures. The corresponding selected area electron diffraction (SEAD) pattern displayed in Fig. 3c confirms the presence of diffraction rings corresponding to XRD results.

3.2.3 FTIR of Cu/TiO₂ NCs

FTIR spectrum of chemically reduced Cu/TiO₂ nanocomposites was obtained between 4000-500 cm⁻¹ which is shown in Fig. 4. From the obtained spectrum shows the presence of various chemical constituents: 3500 cm⁻¹ indicate O-H bond stretching, 2976 cm⁻¹ indicate carbonyl (C=O) bend, 2570 cm⁻¹ indicate (C-C) stretch, 1720 cm⁻¹ indicate Cu-O-C and 625 cm⁻¹ indicate Ti-O stretch for Cu/TiO₂ nanocomposites.

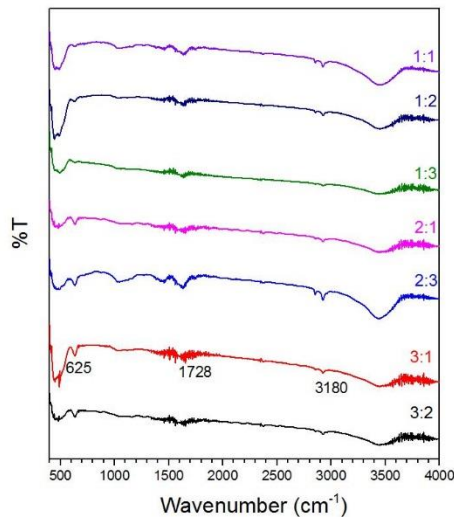


Fig. 4 FTIR spectroscopy of synthesized Cu/TiO₂ NCs

3.2.4 Particle Size Analysis (PSA) of Cu/TiO₂ NCs

The analysis was carried out at 25 °C in a standard mono-dispersive medium maintained at a viscosity of 0.892 mPas. The ND-YAG laser light source (wavelength 532 nm) is used in particle size analyzer. The nanocomposite was dispersed in water for 15 min using ultra sonicator. The dispersed solution was taken into cuvette and observed the distribution of particles. Fig. 5 depicts the almost equal size distribution and the average particles size was 60.9 nm for the synthesized samples. The obtained nanoparticle size distribution of Cu/TiO₂ NCs using PSA is also in good agreement with the HR-TEM results.

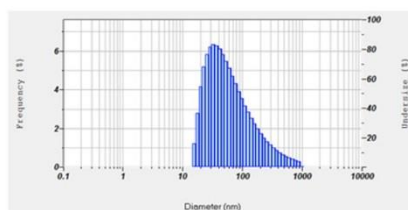


Fig. 5 Size distribution of Cu/TiO₂ NCs analyzed using PSA

3.3 Electrochemical Properties of Cu/TiO₂ NCs

The electrochemical properties of the Cu/TiO₂/GCE NCs were studied by cyclic voltammetry such as in 0.1 M NaOH at the potential range of -1.5 to 1.5 V. From Fig. 6(a) shows the Cu/TiO₂ NCs undergo both reduction and oxidation in cathodic and anodic potentials, respectively. It shows two irreversible reduction peaks in the cathodic region. Restricted potential electrolysis was also accepted out and the experiment reports that each couple corresponds to one-electron transfer process. The oxidation process is irreversible in nature, that the two oxidation peaks are associated with stepwise oxidation process at Cu/TiO₂ NCs. This is because there is a great influence on structural and electrochemical properties of the Cu/TiO₂ NCs, in which Cu acts as good conductor for storage and mobility of electrons and TiO₂ plays as important role in catalytic and fast transfer of electrons, hence this material is best sustainable suitable for antibacterial study.

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From Fig. 6(b) shows there exist two semicircles in the impedance plot. This shows that the system has two time constants, one for the cathode side and another for the anode side. The diameter of each of the semicircle represents the resistance, from this the kinetics i.e., larger resistance value for the cathode than for the anode, which is evident from the diameter of the respective semicircles, this explanation reflects the synthesized Cu/TiO₂ NCs have good conductivity for transfer of electrons for present application.

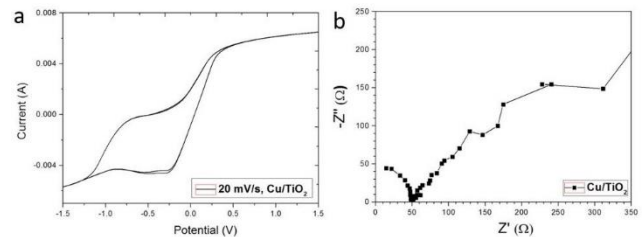


Fig. 6 (a) CVs of Cu/TiO₂/GCE modified electrode at scan rate from 20 mVs⁻¹ (b) electrochemical impedance analysis of Cu/TiO₂/GCE

3.5 Antimicrobial Activity of Cu/TiO₂ NCs

As per the protocol discussed above, the antimicrobial activity of the NCs was carried out by the disc diffusion method. From Fig. 7, the nanocomposites showed both antibacterial & antifungal activity against gram-positive, gram-negative bacteria's and yeast. Control sample which has distilled water, exhibited zero zone of inhibition (Zoi). The cell membranes of the microorganisms interact with the suspension nanocomposite medium. The antimicrobial effect of Cu NPs is due to interactions between metal ions and cells of certain microorganisms such as bacteria and fungus [12–14]. Small size, morphology & high surface area to volume ratio are the factors influencing the antimicrobial effect that allow interacting closely with microbial membranes of each bacterium [15]. By increasing the concentrations of metal oxide nanomaterials there is an increase in cell death, that in-turn affects mitochondrial function, induced lactate dehydrogenase leakage and generated abnormal cell morphology at concentrations as low as 50–100 mg/L [16–19]. The antimicrobial activity of TiO₂ NPs has great potential for antimicrobial agents against microorganisms. Resistance to antimicrobial agents by pathogenic bacteria is a major health problem in recent years. Metal oxides which are electromagnetic in nature have positive charge and whereas microorganisms have negative charge. Interaction between microbes & metal oxide surface leads to oxidation and rapid death of microbes.

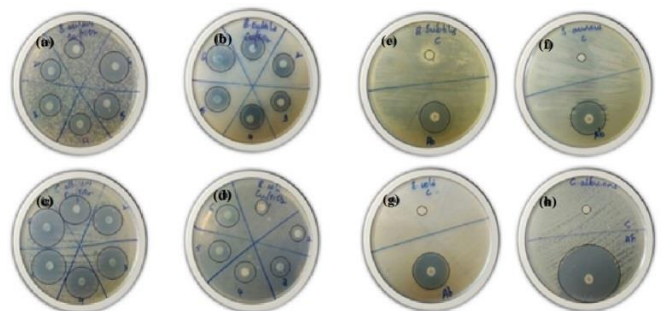


Fig. 7 Cu/TiO₂ NCs inhibiting the growth with respect to (a) *B. subtilis* (b) *S. aureus* (c) *E. coli* (d) *C. albicans* (e-h) inhibitory effect of co-trimazole (standard drug) and control (distilled water) on the growth of *B. subtilis*, *S. aureus*, *E. coli* and *C. albicans*

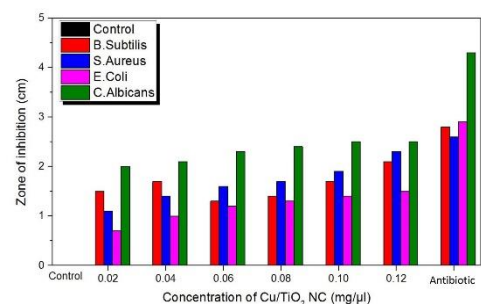


Fig. 8 Bar diagram for the values of Zoi of four species on Cu/TiO₂ NCs

The plates show that Cu based TiO₂ NCs are also showing high antibacterial activity against *S. aureus* i.e., 88.5% inhibition, *B. subtilis* i.e., 75.0%, *E. coli* i.e., 51.7% and *C. albicans* showed relatively high Zoi of 58.1% compared to gram negative ones. Fig. 8 shows in detail the Zoi

measurements as calculated using a Vernier caliper along with their respective control and commercial antibiotic.

A gram positive bacterium like *S. aureus*, *B. subtilis* has 20-80 nm thick cell wall of peptidoglycan (contains amino acids and a sugar-protein shell) where the Cu, Ti ions can easily penetrate. Gram negative bacterium like *E. coli*, has 7-8 nm outer membrane covered with thin cell wall of peptidoglycan [20] where outer membrane prevents the Cu, Ti ions from penetrating. The ions released by nanoparticles attach to the negatively charged bacterial cell wall and ruptures it which leads to protein denaturation and cell death [21] due to the good electrocatalytic activity. Cu based TiO₂ NCs exhibit strong antibacterial activity even in thick layer peptidoglycan and hence is considered as an excellent antibacterial agent, because the combination Cu and TiO₂ make the more suitable for fast electron transfer ability, which reflects in the electrochemical study for the Cu/TiO₂ NC.

4. Conclusion

Copper (Cu) based TiO₂ nanocomposites are in the range of 50-120 nm synthesized through sonochemical route were evidenced by XRD and HR-TEM analysis. The antibacterial activity was correlated with electrochemical studies and estimated the best suitability for antibacterial activity based on the electron transfer and electrochemical properties of Cu/TiO₂ NC. Copper based nanocomposites showed inhibitory effect against gram positive, gram negative strains and yeast. Cu based TiO₂ NCs exhibit strong antibacterial activity even in thick layer peptidoglycan and hence is considered as an excellent antibacterial agent. Finally reported, Cu based TiO₂ NCs are shows high antibacterial activity against *S. aureus* i.e., 88.5% inhibition, *B. subtilis* i.e., 75.0%, *E. coli*, i.e., 51.7% and *C. albicans* showed relatively high Zol of 58.1% compared to gram negative ones. Based on results, we conclude that the copper based TiO₂ nanocomposites also have a high impact on public health issues in drug delivery, anticancer and biomedical applications.

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