4.1 SECTION A-COPPER NANOPARTICLES:

4.1.1 INTRODUCTION:

In the present study, we have prepared tetra alkyl ammonium salts protected copper oxide nanoparticles by electrochemical reduction method. We have also studied the effect of current density, concentration of ammonium salt, and nature of tetra alkyl ammonium salt on the particle size. These synthesized nanoparticles were used for the antibacterial screening against gram negative and gram positive human pathogens.

Copper and copper based materials are employed as antimicrobial agents [16,17]. Realizing the potential applications of copper oxide nanoparticles we have prepared stable copper oxide nanoparticles by electrochemical reduction method and investigated their antibacterial activities using human pathogens like, gram positive and gram negative bacteria. Thus nanoparticles of copper and copper oxide have been extensively investigated for enhancing antibacterial property [18,19].

4.1.2 VISUAL INSPECTION:

The inset of Fig.4.1.2.1 shows sample bottles (1-4) of the colloids of copper oxide nanoparticles after the reaction at different interval of time. After 10 min. the gradual change of color from colorless to deep green is the clear indicative of the formation of copper nanoparticles and after 2 hr. the dark black copper oxide nanoparticles are formed in colloidal form.
Fig. 4.1.2.1. Green colored copper oxide colloidal nanoparticles

4.1.3 UV-VISIBLE SPECTROSCOPY:

Metallic nanoparticles exhibit optical absorption originated from the collective oscillation of the conduction electrons when they are excited by light. It is named as surface plasmon resonance (SPR) and is not observed in bulk metal. The optical response depends upon several factors like local refractive index, the dielectric constant of the medium, morphology (size and shape) and the state of aggregation of the nanoparticles. Any change in these physical features will alter the peak position, intensity optical response [20].

The property most immediately observable for metal nanoparticles dispersions of some metal is their colours [21]. Faraday’s classical gold solution shows even now the clear ruby red colour. Indeed gold, silver and copper nanoparticles all have characteristics colour related with their particle size. Thus for these metals, observation of the UV-Visible spectra can be a useful compliment to other methods in characterizing metal nanoparticles.
The electrochemical synthesis of Cu nanoparticles capped with different capping agents (TEAB/TPAB/TBAB) at 6 and 10 mA/cm$^2$ current density has been carried out in the present study. As the electrochemical reduction proceeds, the change in colour of the reaction mixture with time is monitored. It was seen that between 15 to 20 minutes a change in colour usually took place.

In order to obtain the UV-Visible spectra of the copper nanoparticles about 3.0 ml of the reaction mixture was withdrawn after 20 min. of electrolysis. The spectra were thus recorded for individual samples. The overlay spectra of the copper nanoparticles obtained at 6 and 10 mA/cm$^2$ of current density of each ligand have been presented in Fig.4.1.3. a, b and c.

Fig.4.1.3. (a,b, and c) shows UV-Visible absorption spectra during reduction of the precursor as they transformed into the copper oxide nanoparticles and after ageing times during its preservation under ambient conditions. The absorption bands for copper nanoparticles have been reported to be in the range of 550-700 nm. The copper particles showed a high about 684 nm which gradually changed to yellowish brown indicating copper being oxidized from zero to +2 oxidation state. The sharp peak at 684 nm can be attributed to a narrow size distribution of the particles formed in the solution and confirm the formation of CuO NPs [22]. As an evident from Fig. (a, b and c) the particles showed hardly any change in the absorption spectra even after a month of ageing time, consistent with the highly stable nature of nanoparticles.
**Fig. 4.1.3.a:** Optical absorption spectrum for copper oxide nanoparticles capped with TEAB as a function of current density 6 mA/cm²

**Fig. 4.1.3.b:** Optical absorption spectrum for copper oxide nanoparticles capped with TPAB as a function of current density 6 mA/cm²
Fig. 4.1.3.c: Optical absorption spectrum for copper oxide nanoparticles capped with TBAB as a function of current density 6 mA/cm²

The peaks in the UV-Visible region of the spectra are due to the absorption of light in this region, that are the result of probably two type of transformations namely, ligand metal charge transfer excitation and the excitation of conduction electrons in the metal atoms [23]. Mie [24] and recently Wang [25] have carried out theoretical studies on the dependence of the UV absorption maxima on the size of metal nanoparticles. The general trend found by them is that absorption peak shifts towards longer wavelength as the particle size increases [26] and vica versa.

Reetz [15] has reported that in the electrochemical reduction method for the preparation of the metal nanoparticles, the size of the nanoparticles decreases with the increase in the current density. It can thus be said that the colloidal Cu nanoparticles prepared at a current density of 6 mA/cm² are bigger in size as compared to the colloidal Cu nanoparticles prepared at a current density of 10 mA/cm², which are thus comparatively small in size.
Thus in the present study the colloidal copper nanoparticles prepared at a current density of 6 mA/cm² show the absorption band at 650-700nm in range over the entire visible range. These results have been well correlated to the reports and can thus be said to be confirmed.

4.1.4 FTIR SPECTROSCOPY:

The IR spectrum is used to obtain the structural information. The absorption of each type of bond (N-H, C-H, O-H, C-X, C=O, C-O, C-N, C=N) are regularly found in certain small portions of the vibrational infra red region. A small range of absorption can be defined for each type of bond. Outside this range absorptions are normally due to some other type of bonds. The frequencies at which the functional groups appear in the FTIR spectra have been reported [27-33]. The same are used as reference for comparision and confirmation in the present study.

In the present work, copper nanoparticles are synthesized by the electrochemical reduction method in which tetra alkyl ammonium bromide salts (TEAB, TPAB and TBAB) are used as capping agents. These capping agents are organic in nature and possess C-N, C-H, and C-C bonds, which are IR active. Hence IR spectroscopic study of the prepared samples will provide important information regarding the capping of the nanoparticle samples.

The Figures 4.1.4.(a), (b) and (c) shows the IR spectra of the copper nanoparticles capped with the ligands TEAB, TPAB and TBAB respectively, using a current density of 10 mA/cm².

The Figure 4.1.4. (a) shows the IR spectrum of TEAB capped copper nanoparticles. The peaks at about 2960cm⁻¹ and 2870cm⁻¹ indicate the C-H stretching vibrations. The peak at 1603cm⁻¹ corresponds to the symmetrical (ammonium ion) bending. The frequency corresponding to 1472cm⁻¹ indicates the C-H bending. The C-N
linkage in R₄N⁺ ion gives medium band at 1350 cm⁻¹ and 1090 cm⁻¹ which are due to the C-N stretching vibration, all the mentioned frequencies in the IR spectra of the prepared sample confirm the strong adsorption of TEAB on the surface of the copper nanoparticles. The spectrum contains distinct bands at 423 cm⁻¹, 454 cm⁻¹, 515 cm⁻¹, 805 cm⁻¹ and 875 cm⁻¹ which are due to lattice vibration of the mixed phase of Cu and Cu₂O.

The IR spectrum in Figure 4.1.4 (b) of copper nanoparticles capped with TPAB as capping agent shows peaks at 2956 cm⁻¹ indicating the C-H stretching. The frequencies corresponding to 1591 cm⁻¹ and 1464 cm⁻¹ are due to symmetrical (ammonium ion) bending and C-H bending respectively. The unconjugated C-N linkage gives medium bands at 1376 cm⁻¹ and at 1264 cm⁻¹. The mixed phase of copper and copper oxide is characterized by the peaks at 499 cm⁻¹, 806 cm⁻¹ and 1079 cm⁻¹.

Figure 4.1.4 (c) shows the IR spectrum of copper nanoparticles capped with TBAB using a current density of 10 mA/cm². The spectra revels peaks at 2956 cm⁻¹ correspond to the C-H stretching vibrations. The ammonium ion bending is seen at the corresponding absorbance band at 1462 cm⁻¹. The frequency corresponding to 1593 cm⁻¹ relates to the H-C-H bending vibrations. The C-N linkage in R₄N⁺ ion gives medium bands at 1056 cm⁻¹ due to the C-N stretching vibrations. The IR spectra shows distinct bands at 1380 cm⁻¹ and 1262 cm⁻¹, which are due to the carbon dioxide and nitrate like impurities absorbed from atmospheric air during the storage. The spectrum also contains distinct peaks at 413 cm⁻¹, 476 cm⁻¹ and 804 cm⁻¹ which correspond to the mixed phase that contains copper and copper oxide. The values for the mixed phase have been reported [34-37].

Thus the FTIR study of copper nanoparticles capped with different capping agents like TEAB, TPAB and TBAB reveals that the capping agents are adsorbed on the surface of the nanoparticles forming a
protective layer. The capping agents also help to prevent the agglomeration of the nanoparticles thereby controlling the size of the nanoparticles.

**Fig. 4.1.4. (a)** IR Spectrum of CuO nanoparticles using 0.01 M TEAB

**Fig. 4.1.4. (b)** IR Spectrum of CuO nanoparticles using 0.01 M TPAB
4.1.5 X-RAY DIFFRACTION PATTERN:

In order to understand the phase symmetry of the prepared copper oxide nanoparticles a systematic study on the XRD was undertaken. Fig. 4.1.5. shows XRD pattern for CuO nanoparticles. The lattice parameter observed $a = 4.653$, $b = 3.410$ $c = 5.108$ at alpha = 90°, beta = 90° and gama = 90°. Sharp peaks were obtained corresponding to the planes (111), (201), (211) and (312) indicates the monoclinic structure of CuO nanoparticles [38,39] and which was found to be highly crystalline in nature. The diffraction is in good co-ordination with ASTM card no. 74-1021. The average particle size was calculated to be 9.56 nm using Debye Scherrer formula.

Fig. 4.1.4. (c) IR Spectrum of CuO nanoparticles using 0.01 M TBAB
Fig. 4.1.5.1. X-ray diffraction pattern of Copper oxide nanoparticles capped with TBAB at room temperature

Table 4.1.5.1. XRD analysis data for copper oxide nanoparticles

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<th>k</th>
<th>l</th>
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<th>$2\theta$° (Cal)</th>
<th>d(Å) (Exp)</th>
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<th>Intensity (Exp)</th>
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<td>77.133</td>
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4.1.6 SCANNING ELECTRON MICROSCOPY:

The scanning electron microscope is a type of electron microscope that images the sample surface by scanning it with high energy beam of electrons. Some incident electrons may be scattered or reflected out of the sample with no change in energy. The fraction of incident electrons thus reflected from specimen is called back scattering coefficient and this parameter varies with accelerating voltage and atomic number of sample. Thus if SEM is operated so as to produce an image using back scattered electrons, it should be noted that resolution is considerably reduced in back scattered mode of operation, because back scattered electrons are generated in a large volume of sample (1-3 nm deep). In this study, it is necessary to consider not only intrinsic instrument resolution but also interaction of the incident electron beam and the sample.

The evaluated morphology of copper nanoparticles capped with TEAB TPAB and TBAB by SEM is shown in Fig. 4.1.6.1, Fig. 4.1.6.2 and Fig. 4.1.6.3 respectively. The scanning electron microgram and the particle size distribution for the sample of copper nanoparticles capped with TEAB, TPAB and TBAB at current density of 6 mA/cm² is obtained for various resolutions.

Fig.4.1.6.1 (a) and (b) SEM microstructure of the electrochemical reduction derived copper oxide nanoparticles reveals the presence of dense agglomerations Fig. 4.1.6.1 (a) Shows these particles have irregular shape and their distribution is not uniform. This is probably due to the partial solubility of the surfactant in the solvent under the given experimental conditions. Fig. 4.1.6.2 and Fig. 4.1.6.3 shows the presence of porous nanoparticles that are agglomerated irregularly. For the purpose of identifying or comparing the shapes of the copper nanoparticles a number of reports [40-42] on the SEM micrographs of
copper nanoparticles were studied. However such shape or surface could not be found in any of the reference.

**Fig. 4.1.6.1** (a) and (b) SEM of as prepared copper oxide nanoparticles capped with 0.01M TEAB at 6mA/cm²

**Fig. 4.1.6.2** SEM of as prepared copper oxide nanoparticles capped with 0.01M TPAB at 6 mA/cm²

**Fig. 4.1.6.3** SEM of as prepared copper oxide nanoparticles capped with 0.01M TBAB at 6 mA/cm²
4.1.7 ENERGY DISPERSIVES ANALYSIS:

Copper nanoparticle sample was analyzed qualitatively and quantitatively by EDS. Fig. 4.1.7.1 shows that copper, oxygen and bromine are present in the sample and its composition is given in Table 4.1.7.1. According to the atomic % and mass % of elements clearly shows that the copper oxide nanoparticles are formed. Br is present in trace amount because of TBAB used as capping agent.

![EDS spectrum of Cu nanoparticles](image)

**Fig. 4.1.7.1** EDS spectrum of Cu nanoparticles

**Table 4.1.7.1** EDS analysis of Cu nanoparticles

<table>
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<th>Constituents</th>
<th>Mass (%)</th>
<th>Atom (%)</th>
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</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>O</td>
<td>14.94</td>
<td>32.38</td>
</tr>
<tr>
<td>Br</td>
<td>1.05</td>
<td>0.46</td>
</tr>
</tbody>
</table>

4.1.8 (A) TRANSMISSION ELECTRON MICROSCOPY:

Transmission electron microscopic study has been done for evaluates, particle shape and size distribution of copper oxide nanoparticles as shown in Fig. 4.1.8 (A)1 and 2 synthesized with capping agents TBAB and TPAB respectively. Micrograph also showed
particles of various sizes, small and big and some clusters have been observed in both Fig.4.1.8.1 (A and B). In fig. 4.1.8.1 (b) clear anisotropy is observed with the particles in size range of less than 5nm and greater up to about 10nm which is resembled to the XRD results.

4.1.8.1 (A) TEM micrograph of copper oxide NPs capped with 0.01M TBAB (current density 6 mA/cm²)

4.1.8.2 (A) TEM micrograph of copper oxide NPs capped with 0.01M TPAB (current density 6 mA/cm²)
4.1.8 (B) HR-TRANSMISSION ELECTRON MICROSCOPY:

Transmission electron microscopy was used to analyze the size and electron density of the metal nanoparticles recorded from the drop coated films of the metal nanoparticles synthesized using 0.01 M TBAB in THF and Acetonitrile.

All the micrographs of copper oxide nanoparticles clearly shows that the uniform size distribution of the Cu NPs and they are spherical in shape.

The HR-TEM analysis shows that the copper oxide nanoparticles have an average size in the range of 4.0 nm to 20 nm.

4.1.8 B (a,b,c) HR-TEM micrograph of copper oxide NPs capped with 0.01M TBAB (current density 6 mA/cm²) (d) SAD Pattern for copper oxide NPs
The results obtained from the TEM study are found to be co-related and thus authenticated with the XRD pattern analysis for the same samples. The results of TEM analysis and XRD analysis show identical trends and thus validate the findings.

4.1.9 X-RAY PHOTOELECTRON SPECTROSCOPY:

X-ray Photoelectron Microscope (XPS) is a powerful technique for the study of transition metal compound having localized valence ‘d’ orbital and also useful to obtain important information about the surface molecule and electronic structures.

In CuO, copper exists in the divalent state having mainly d\(^0\) character. However, Cu\(_2\)O is expected to have an essentially full Cu 3d shell [43]. Fig. 4.1.9.1 shows the wide XPS picture of the Cu nanoparticles capped with tetra butyl ammonium bromide (TBAB) at a current density 6 mA/cm\(^2\). The C1s peak lies at 297.0eV which should be corrected to 284.6eV. All the other peaks are corrected accordingly. No peaks of other elements except C, Cu and O were observed in the picture, indicating the high purity of the product.

The high resolution XPS spectrum (Fig. 4.1.9.2) shows two peaks that lie at 933.5 and 953.1 eV and are assigned to Cu (2\(P_3/2\)) and Cu (2\(P_1/2\)) respectively. In Fig. 4.1.9.3 the broad peak at 533 eV is attributed to O 1s. The results are in agreement with the literature data [44].
4.1.9.1 XPS spectrum of wide CuO nanoparticles capped with TBAB at 6 mA/cm² current density

**Fig. 4.1.9.2** XPS spectrum of Cu 2P in CuO nanoparticles capped with TBAB at 6 mA/cm² current density
**Fig. 4.1.9.3** XPS spectrum of O1s in CuO nanoparticles capped with TBAB at 6 mA/cm² current density

The atomic ratio of Cu:O calculated on the basis of Cu2P and O1s spectra is approximately equal to 3:4 which shows that the surface of the product is rich in Oxygen. The XPS and EDS spectra of CuO nanoparticles are identical with those of CuO nanoparticles.

### 4.1.10 ANTIBACTERIAL ACTIVITIES OF COPPER OXIDE NPs

Antimicrobial activities of the synthesized copper oxide nanoparticles are performed against human pathogens like gram positive and gram negative bacteria by following the paper disc plate method [70]. Control experiments were also carried out in the presence of ampicillin. The solvents ACN and THF does not show any bacterial activity. The 10mL nutrient agar-agar medium containing 50 μg/mL-100 μg/mL copper oxide nanoparticles were prepared. Each set was inoculated aseptically with 10μL of respective bacterial suspension (approximately 108 CFU/mL). The inoculated sets were incubated at 37°C for 24h. The experiments were carried out in
triplicates. Viable bacterial colonies were counted and recorded by naked eye. Determining the lowest concentration that locked bacteria growth as the MIC, tests were performed three times for each strain to calculate the value as the final MIC value. The counts on three plates corresponding to a particular sample were averaged. In other experiments interaction between bacteria and copper oxide nanoparticles was studied in agar-agar medium for 1h in the presence of nanoparticles and then sediment the bacteria and reculturing them in fresh medium (without nanoparticles).

Bacterial cell size usually ranges in micron range. These cells have cellular membranes which contains pores in nanometer range. The nanoparticles which were synthesized have a size less than that of pore size in the bacteria and thus they have a unique property of crossing the cell membrane without any hindrance.

The zone of inhibition shown by CuO NPs. against gram positive and gram negative bacteria were shown in Table 4.1.1.8.1. The data clearly shows that copper oxide nanoparticles acted as excellent antibacterial agents against both gram positive and gram negative bacteria. The antibiotic amphicillin is more effective against gram positive than gram negative bacteria.
Table 4.1.10.1.: *In vitro* antibacterial screening of synthesized CuO NPs

<table>
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<th>AN + THF (4:1)</th>
<th>Amphicillin</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
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<table>
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<tr>
<th>Zone of Inhibition (mm)</th>
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<th>11</th>
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<td>16</td>
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<td>00</td>
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<tr>
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<td>19</td>
<td>23</td>
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</table>

Antibacterial testing for antibacterial activity revealed that copper oxide nanoparticles acted as excellent antibacterial agents against both gram positive and gram negative bacteria.