RESULTS AND DISCUSSION

4.1. Electrochemical Synthesis of Copper oxide Nanoparticles

Electrochemical process a soft chemical technique has been used for the synthesis of copper oxide nanoparticles in different media. The control of particle formation is a difficult process and demanding understanding of interactions between the reagents and other parameters (electrode, electrolyte, temperature, electrolysis time, current, solvent, solution and dimension or shape of the cell) affecting the particle. It has been observed that the shape, size and yield of nanoparticles are highly affected by different reaction parameters as shown in Table 4.1.1.

Various reaction parameters have been optimized for the synthesis of copper oxide nanoparticles. These reaction parameters are discussed as follow:

4.1.1. Effect of Electrolytes

In the electrochemical route, the bulk metal is oxidized to metal cations, which migrate to the cathode where the reduction occurred. The effect of ion-solvent interaction on the conductivity of an electrolyte has been well reported in the literature (Szejgis et al., 2012). The electrical conductivity of an electrolyte solution gave many important qualitative insights (Chatterji et al., 2006). The effect of different supporting electrolytes such as sodium hydroxide, sodium nitrate and sodium carbonate has been studied at constant current and solvent system for the formation of CuO NPs. The supporting electrolytes (participate in an electrode process by attacking intermediate species or alter product distribution by changing the acid-base character of the solution) stabilized the growth of the particles and also increased the rate of the reaction (Ellah et al., 2013). The effect of the supporting electrolyte on controlling the morphologies of CuO NPs suggested that the ion diffusion of electrolyte played a key role for growth mechanism. In case of NaOH electrolyte, the solution change gradually from colorless liquid to light yellow suspension and finally dark bluish brown precipitates. It was due to transformation at anode from copper to copper oxide particles through several reactions as \( \text{Cu} \rightarrow \text{Cu}^+ \rightarrow \text{Cu(OH)}_2 \rightarrow \text{CuO} + \text{Cu}_2\text{O} \). The formation of CuO and Cu₂O was further confirmed by XRD and TEM as shown in Figures 4.1.1 - 4.1.3. While in case of NaNO₃ and Na₂CO₃, the color changes from colorless to bluish brown suspension and
finally dark bluish brown. The yield of product was observed higher in sodium carbonate as compared to other electrolytes.

4.1.2. Effect of Current

The effect of current on the shape and size of CuO NPs were studied in NaOH (1.25 mM) electrolyte and water-ACN (12:1) solvent system (Figure 4.1.4). To study the effect of current onto the shape and size of nanoparticle, a series of preparations was carried out by varying the current (20, 50 and 100 mA). During the electrolysis, a change in current affects the yield of product and color of the solution. It has been observed that the yield of product increased with the increases in current. It was due to increase in the rate of hydrogen evolution with current, which increased the penetration and distribution of hydrogen bubbles on copper surface (Ali et al., 2008). At 20 mA, solution takes 10 min to turn into light yellow color and finally to light bluish suspension after 2 hours. When, 50 mA current was applied to electrolyte solution, it takes few minutes to turn into light blue color suspension. At 100 mA current, the color of solution immediately changed to dark bluish brown suspension. It has been observed that the particle size decreased with increase in current. It was due to correlation between current and particle size obtained from the free energy of formation of nanoparticle (Ali et al., 2008). TEM results clearly showed the effects of current on the particle size of CuO NPs as shown in Figure 4.1.4.

4.1.3. Effect of Electrolysis Time

CuO NPs has been prepared at 100 mA current in water-ACN (12:1) solvent and NaOH (1.25 mM) electrolytes were studied for different electrolysis times (30, 60 and 120 min) at room temperature. The results of electrolysis times on the size of particle were shown in Figure 4.1.5. It has been indicated that with increase in the electrolysis time from 30 to 120 min the yield of product and size of particle increased (Goh et al., 2011). It was due to the increase in the number of nuclei formation at cathode.

4.1.4. Effect of Solvent

The effect of different solvent such as distilled water, water-methanol and water-acetonitrile has been investigated on the shape and size of CuO NPs. All the reactions performed at 100 mA current in a NaOH (1.25 mM) supporting electrolyte. Acetonitrile were a polar aprotic solvent, due to their high conductivity, solvating power, high dielectric constant (ε = 37) and nontoxicity have used as solvent for the
fabrication of metals and alloys (Seo et al., 2013). In deionized water solvent light greenish blue suspension was formed. The solution turned dark yellow suspension in presence of water-methanol solvent. In water-ACN solvent, the solution changes from colorless to light yellow suspension and finally dark bluish brown precipitates were formed. The yield of the product was recorded higher in water-ACN solvent followed by water-methanol and water solvents. The change in shape and size of CuO NPs in different solvent media are shown in Figure 4.1.6. Based on the above results, the possible mechanisms for synthesis of CuO NPs have been presented as follow (Yuan et al., 2007):

**In presence of NaOH electrolyte**

Anode:

\[ \text{Cu} \rightarrow 2\text{Cu}^{2+} + 2e^- \]

Cathode:

\[ \text{OH}^- + \text{H}_2\text{O} + 2e^- \rightarrow 2\text{OH}^- + \text{H}_2 \]

Electrolyte solution:

\[ 2\text{Cu}^{2+} + 2\text{OH}^- \rightarrow \text{CuO} + \text{Cu}_2\text{O} \]

**In presence of NaNO\textsubscript{3} electrolyte**

Anode:

\[ \text{Cu} \rightarrow 2\text{Cu}^{2+} + 2e^- \]

Cathode:

\[ \text{NO}_3^- + \text{H}_2\text{O} + 2e^- \rightarrow 2\text{OH}^- + \text{NO}_2^- \]

Electrolyte solution:

\[ 2\text{Cu}^{2+} + 2\text{OH}^- \rightarrow \text{CuO} + \text{Cu}_2\text{O} \]

**In presences of Na\textsubscript{2}CO\textsubscript{3} electrolyte**

Anode:

\[ \text{Cu} \rightarrow 2\text{Cu}^{2+} + 2e^- \]

Cathode:

\[ \text{CO}_3^{2-} + \text{H}_2\text{O} + 2e^- \rightarrow 2\text{OH}^- + \text{CO}_2 \]

Electrolyte solution:

\[ 2\text{Cu}^{2+} + 2\text{OH}^- \rightarrow \text{CuO} + \text{Cu}_2\text{O} \]
In the presence of current, OH\(^-\) ions and Cu\(^{2+}\) ions are generated on the surface of cathode and anode at the same time. Cu\(^{2+}\) ion reacts with OH\(^-\) ions in the reaction mixture to produce CuO and CuO\(_2\). It has been observed that the nature of electrolyte affects the shape and size of particles due to presence of different anions.

**4.1.5. Characterization Techniques**

**4.1.5.1. XRD Analysis**

Figure 4.1.1 shows the XRD spectra of CuO NPs synthesized in different electrolyte at different temperature. Figure 4.1.1a-c showed the XRD pattern of CuO NPs prepared in SH, SN and SC electrolytes. The diffraction lines observed were broad and less intense. The diffraction peaks appeared at 20 angles of 32.5°, 35.6°, 36.6°, 38.7°, 48.8°, 50.5°, 58.0°, 61.6°, 66.1°, 68.6° and 74.1° corresponds to CuO and Cu\(_2\)O NPs. The broad and less intense diffraction peaks at 20 values of 32.6°, 35.7°, 36.7°, 38.8°, 42.6°, 48.9°, 53.6°, 58.0°, 61.7°, 66.2°, 68.0° and 72.2° were recorded for the nanoparticles in SN electrolyte as shown in Figure 4.1.1b. Figure 4.1.1c shows the CuO NPs prepared in SC electrolyte with 20 angle of 29.8°, 33.1°, 35.6°, 36.7°, 38.6°, 42.7°, 43.4°, 49.1°, 50.5°, 53.3°, 57.9°, 61.8°, 66.7°, 68.0° and 74.1°. The size of CuO particles were calculated from XRD data using Debye Scherrer equation. The mixed phase of CuO and Cu\(_2\)O was observed in all the cases at room temperature (Farbod et al., 2014). X-ray diffraction peaks were indexed with lattice planes and compared to the International Center for Diffraction Data (ICDD) Card No: 41-0254 (Cheng et al., 2012). The growth mechanism of electrodeposited CuO NPs was reported in the literature (Yuan et al., 2007). XRD patterns of sample calcined at higher temperature corresponds to broad diffraction peaks, indicated the formation of CuO with smaller particle.

The XRD pattern of copper oxide NPs prepared in different electrolytes after calcination is shown in Figure 4.1.1a-c. The two main peaks of CuO at 35.6° and 38.7° were observed in SH, SN and SC electrolyte (Bai et al., 2012). The crystallite average grain size of copper oxide in SH, SN and SC electrolyte at 300 °C were recorded as 13 nm, 16 nm and 20 nm, respectively. At high calcinations temperature of 600 °C, characteristic peak of CuO was appeared in all the samples.
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Figure 4.1.1: XRD spectrum of CuO NPs prepared in presence of (a) sodium hydroxide (b) sodium nitrate (c) sodium carbonate

The average particles size obtained at 600 °C were 16 nm, 15 nm and 19 nm, respectively in SH, SN and SC electrolytes. With further increase in calcination temperature from 600 °C to 900 °C, the characteristic CuO peaks become sharper and crystalline. The average particles size obtained in SH, SN and SC electrolyte were 26 nm, 27 nm and 30 nm, respectively.

4.1.5.2. TEM Analysis

TEM micrograph of CuO NPs prepared in different reaction conditions are shown in Figure 4.1.2 - 4.1.5. TEM results indicated that all reaction conditions not only affect the particle size but also influenced the shape of particles. The well dispersed round shape particles was found in the presence of SH electrolyte without calcination.
Needle shaped and agglomeration of particle was observed in presence of SN and SC electrolytes (Figure 4.1.2a-c) (Yuan et al., 2007). The good agreement between particle size and crystal size indicated the highly crystallinity of the particles. TEM images of CuO NPs prepared in different electrolyte at various calcination temperatures of 300 °C and 900 °C are shown in Figure 4.1.3. The round shaped particles with average particle size of 20 nm and 25 nm were obtained in SH and SC at 900 °C. The needle shaped particles were recorded (200-500 nm at 900 °C) in SN as revealed from Figures 4.1.3d-e. The selected area electron diffraction (SAED) pattern of CuO NPs indicated the crystalline and ordered orientations (Figure 4.1.3c). The SAED pattern showed the rings with diffraction pattern ascribed to (111), (111), (202), (020), (113), (310) and (220), which confirmed the monoclinic crystalline structure with lattice parameters as a = 4.685 Å, b = 3.423 Å, c = 5.132 Å, β = 91.52 and V=78.70 Å (Cheng et al., 2012). The micrographs showed that calcinations temperature and electrolyte strongly influenced the morphology and size of nanoparticles.

Figures 4.1.4a-b and Figure 4.1.3 b shows TEM micrographs of CuO NPs prepared in presence of SH electrolyte and water-ACN solvent. The results revealed that the average particle size of CuO NPs were 60 nm, 50 nm and 20 nm at currents of 20 mA, 50 mA and 100 mA, respectively. TEM histogram showed that the particle size decreased with the increase in the current. The obtained results were in well agreement with reported literature (Yuan et al., 2007).

Figures 4.1.5a-c shows the TEM micrographs of CuO NPs (SH and water-ACN at 100 mA current) under the influence of electrolysis time. The images clearly indicated incomplete formation of particle prepared at 30 min and the particle size increased with reaction time.

4.1.5.3. SEM Analysis

Figure 4.1.6 displays the SEM images in different solvents such as water, water-methanol and water-acetonitrile. The granular spherical shape particles were obtained in the presence of water and rough surface in presence of water-methanol solvent. Water-ACN solvent resulted small granular spherical shape particles with aggregation.
Figure 4.1.2: TEM micrographs of CuO NPs prepared in presences of different supporting electrolytes (a) sodium hydroxide (b) sodium nitrate (c) sodium carbonate
Figure 4.1.3: TEM micrographs of CuO NPs in presences of sodium hydroxide, sodium nitrate, sodium carbonate supporting electrolytes calcined at (a-d) 300 °C (b) (e-h) 900 °C (c) (f-i) electron diffraction pattern and particle size distributions of CuO NPs
Figure 4.1.4: TEM micrographs of CuO NPs at current (a) 20 mA (b) 50 mA
Figure 4.1.5: TEM micrographs of CuO NPs at different electrolysis time (a) 30 min (b) 60 min (c) 120 min
Figure 4.1.6: SEM micrographs of CuO NPs prepared in the presence of (a) water (b) water-methanol and (c) water-ACN solvent (d) EDX of CuO NPs
4.1.5.4. EDX Analysis

The composition of the CuO NPs was determined by energy dispersive X-ray spectroscopy. EDX spectrum of CuO NPs indicated the existence of carbon, copper and oxygen as shown in Figure 4.1.d.

4.1.5.5. UV-visible Analysis

CuO NPs band gap was determined by recorded the UV spectra in the range from 200 nm to 900 nm as shown in Figure 4.1.7. The absorption peak at 260 nm confirmed the formation copper oxide nanoparticles. The optical band gap of CuO NPs is calculated using the Tauc’s relation as (Ray et al., 2001):

\[ \alpha h\nu = A(h\nu - E_g)^n \]

where A is constant (independent of n), \( \alpha \) is absorption coefficient and n is the exponent that depends upon the quantum selection rules.

A plot of \((\alpha h\nu)^2\) against photon energy (hv) (Figure 4.1.7b) showed straight line due to direct allowed transition (n = 1). The optical band gap of CuO NPs was calculated to be 1.52 eV. It may be due to the quantum size effect in case of CuO NPs (Yuan et al., 2012).

4.1.5.6. FTIR Analysis

Figure 4.1.7c shows the FTIR spectra of CuO NPs prepared in SH and water-ACN at 100 mA current. A weak band at around 2318 cm\(^{-1}\) may be due to the vibration of C-O band (Sadollahkhani et al., 2014). The peaks at 587 cm\(^{-1}\) and 536 cm\(^{-1}\) may be due Cu-O bond stretching (Frost et al., 2007; Mushtag et al., 2010). The presence of peaks in the region between 500 nm to 600 nm indicated the formation of CuO NPs.

4.1.5.7. TGA Analysis

Figure 4.1.8 shows the thermogravimetric analysis of CuO NPs. The initial weight loss of about 2 % was observed up to 200\(^\circ\) C. It may be due to the dehydration. The weight loss of only 3.3% was recorded in between 200-400\(^\circ\) C. It may be due to complete oxidation of compound (Ortiz et al., 2004). It was revealed that no weight
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Figure 4.1.7: (a) UV-Visible spectra of CuO NPs (b) plot of $(\alpha h\nu)^2$ vs $h\nu$ of CuO NPs (c) FTIR spectra of CuO NPs

Figure 4.1.8: TG/DTA of CuO nanoparticles
loss was observed from 400°C to 600°C. The results inferred the high stability of CuO NPs as only 5.3% weight loss occurred up to 600°C.

### 4.1.6. Applications of Copper Oxide Nanoparticles (CuO NPs)

CuO NPs prepared in water-ACN solvent, SH electrolyte, 100 mA current, 120 min calcined at 300°C has been selective for photocatalytic and antimicrobial activity. The photocatalytic degradation of different dyes such as methylene blue (MB), methyl red (MR) and congo red (CR) has been attempted. The structures of organic dyes are shown in Scheme 4.1:

![Scheme 4.1: The chemical structure of MB, MR and CR dyes](image-url)
4.1.6.1. Photocatalytic Degradation of Dyes

Photodegradation of cationic dyes such as MB, MR and CR using CuO NPs under sunlight irradiation were explored. The decrease in absorbance band intensities in presences of CuO NPs with irradiation time was observed for MB, MR and CR as shown in Figure 4.1.9. The results revealed the efficient degraded of different dyes. The photodegradation of different dyes in the absence of CuO NPs was observed only 2 to 2.28 % as shown in Figure 4.1.9b. It indicated very low self-sensitized photolysis of dyes. The absorbance decreased gradually with exposure time for all dyes in presence of CuO NPs. The color of dyes solution faded within 120 min of sunlight irradiation in presence of CuO NPs. The degradation of 81 %, 81% and 67% was recorded for MB, MR and CR dyes, respectively within 80 min of photoirradiation. While the degradation of 93%, 90% and 85% was observed for MB, MR and CR dyes, respectively after 120 min of photolysis as shown in Figure 4.1.9c. The degradation of methylene blue was observed higher as compared to other organic dye. When dyes solution was exposed to light, the aggregate species disappear first followed by the monomeric ones. The results inferred that photodegradation destroys not only the conjugate system but also the intermediate products (Guettai et al., 2005). It is also revealed that the chemical structure of MB and MR was found more susceptible to oxidation as compared to CR. The rate of photo catalytic degradation for CR (0.01749 min\(^{-1}\)) was recorded lower than MB (0.02059 min\(^{-1}\)) and MR (0.02046 min\(^{-1}\)). It may be due to the large steric hindrance arising from biphenyl group and naphthenic groups (Lachheb et al., 2002).

When CuO NPs were irradiated by sunlight the electron (\(e^{-}_{CB}\)) and holes (\(h^{+}_{VB}\)) are generated on CuO NPs surface. The holes counter with water adhered to the surfaces of CuO NPs to form highly reactive hydroxyl radicals (’OH). In the intervening time, oxygen acted as an electron acceptor by forming a superoxide radical anion (O\(_{2}^{-}\)). The dyes has been destroyed through direct oxidation by the ’OH radicals and O\(_{2}^{-}\)-radicals. The mechanism for catalytic degradation of dyes using CuO NPs in existence of sunlight irradiation was explained as follow (Zeng et al., 2009):
\[
\text{CuO} + \text{hv} \rightarrow \text{CuO} (e^-_{\text{CB}} + h^+_{\text{VB}})
\]

\[
\text{CuO} (h^+_{\text{VB}}) + \text{H}_2\text{O} \rightarrow \text{H}^+ + \cdot \text{OH}
\]

\[
\text{CuO} (e^-_{\text{CB}}) + \text{O}_2 \rightarrow \text{O}_2^-
\]

\[
\text{O}_2^- + \text{H}^+ \rightarrow \text{HO}_2^-
\]

Dye + \cdot \text{OH} \rightarrow \text{degradation products}

Dye + (h^+_{\text{VB}}) \rightarrow \text{oxidation products}

Dye + (e^-_{\text{CB}}) \rightarrow \text{reduction products}

Figures 4.1.9d-f show the plot of ln A vs irradiation time for MB, MR and CR. The linear correlation with good precision was observed. It confirmed the pseudo-first-order rate order kinetics for dyes degradation. The constant rate value clearly specified the fast and effective photodegradation of MB (Sadollahkhani et al., 2014).

4.1.6.2. Antimicrobial Activity of CuO NPs

The antimicrobial activity of CuO NPs was studied against \textit{E. coli}, \textit{S. aureus}, \textit{A. nigres} and \textit{C. albicans} strain using growth curve method. Figure 4.1.10 shows the growth curves of microbes at altered concentration of CuO NPs. The maximum growth of microbes was inhibited at 50 µg/mL after 16 hours period of incubation in all the cases. The 94% inhibition of \textit{A. nigres} was recorded using CuO NPs. It may be due to the binding of NPs to the outer membrane of microbes which resulted in inhabitation of cell wall, nucleic acids synthesis, active transport 30s ribosomal subunit and finally leads to the demise of the cell (Gupta et al., 2015).
Figure 4.1.9: (a) Photodegradation of MB, CR and MR with CuO NPs (b) without CuO NPs under sunlight (c) % of degradation of dyes (d) plot of ln A versus time for MB (e) CR and (f) MR dye
Figure 4.1.10: Growth curves of (a) *E. coli* (b) *S. aureus* (c) *C. albicans* and (d) *A. nigres* exposed to 25 µg and 50 µg concentration (mg/mL) of CuO NPs (e) inhibition rate (%) CuO NPs against microbes
Table 4.1.1. Preparation of CuO NPs at different experimental conditions

<table>
<thead>
<tr>
<th>Reaction parameters</th>
<th>Solvents</th>
<th>Electrolytes</th>
<th>Current</th>
<th>Reaction time</th>
<th>Stirring rate (r/s)</th>
<th>TEM (Average particle size (nm))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of electrolyte</td>
<td>Water-ACN</td>
<td>NaOH</td>
<td>100 mA</td>
<td>2 h</td>
<td>5</td>
<td>20</td>
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<td>100 mA</td>
<td>2 h</td>
<td>5</td>
<td>200</td>
</tr>
<tr>
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<td>Water-ACN</td>
<td>Na₂CO₃</td>
<td>100 mA</td>
<td>2 h</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>Effect of solvent</td>
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<td>100 mA</td>
<td>2 h</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Water-methanol</td>
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<td>100 mA</td>
<td>2 h</td>
<td>5</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Water-ACN</td>
<td>NaOH</td>
<td>100 mA</td>
<td>2 h</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
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<td>20 mA</td>
<td>2 h</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Water-ACN</td>
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<td>2 h</td>
<td>5</td>
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<tr>
<td>Effect of electrolysis time</td>
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<td>30 min</td>
<td>5</td>
<td>Incomplete formation of particle</td>
</tr>
<tr>
<td></td>
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<td>100 mA</td>
<td>60 min</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td></td>
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<td>NaOH</td>
<td>100 mA</td>
<td>120 min</td>
<td>5</td>
<td>20</td>
</tr>
</tbody>
</table>
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Results and Discussion

4.2. Electrochemical Synthesis of Aluminum oxide Nanoparticles (Al$_2$O$_3$ NPs)

Electrochemical synthesis of Al$_2$O$_3$ NPs has been influenced by number of reaction parameters such as nature of electrolyte, electrode, solvent, temperature, dimension of cell, current etc. The effect of these reaction parameters on the synthesis of aluminum oxide nanoparticles are discussed as follow:

4.2.1. Effect of Electrolyte

Electrochemical synthesis depends upon the nature of supporting electrolyte. The supporting electrolytes changed the acid-base character of the solution and participate by attacking intermediate species, alter the product distribution, stabilized the growth of the solution and increased the rate of reaction (Huang et al., 2006). During electrochemical process, bulk aluminum at anode was oxidized as aluminum ions which migrated to cathode where reduction occurred with the formation of adatoms (Huang et al., 2006). The effect of different supporting electrolytes such as sodium hydroxide, sodium nitrate and sodium carbonate has been studied at constant current, electrolysis time and solvent system. It has been observed that the electrolyte solution takes few minutes to change the color from colorless to light milky white suspension. It may be due to the transformation of aluminum into Al$_2$O$_3$ particles through several reactions as Al $\rightarrow$ Al$^{3+}$ $\rightarrow$ Al(OH)$_2$ $\rightarrow$ Al$_2$O$_3$. It was further confirmed from XRD and TEM results as shown in Figures 4.2.1- 4.2.4. The yield of nanoparticle was observed higher in presences of SC as compared to SH and SN electrolytes.

4.2.2. Effect of Current

During the electrochemical reaction the current flow through the electrolyte as ions moving from anode to cathode depends upon the applied electrical field. The effect of current was carried out at 20 mA, 50mA and 100 mA current in water-ACN (12:1) solvent, NaOH (1.25 mM) supporting electrolyte. During the electrolysis, the change in current affected visibly color of solution. At the highest current of 100 mA, the color of solution turned milky white immediately. Fast generation of ions occurred at anode. At cathode the rate of hydrogen evolution and increased the penetration distribution of hydrogen bubbles (Ali et al., 2008). At the 20 mA, generation of ion
was slow and the color of the solution changed only slightly. It was observed that the yield of product was increased with the increase of current. It has been observed that the high nucleation rate decreased the particles size (Figure 4.2.5). The change in particles size might be attributed to correlation between the current and particle size obtained from the free energy of formation of nanoparticle (Ali et al., 2008).

4.2.3. Effect of Electrolysis Time

Al₂O₃ NPs prepared in water-ACN (12:1) solvent, NaOH (1.25mM) supporting electrolytes at 100 mA current were studied for different electrolysis times (10, 30 and 60 min) at room temperature. It has been observed that with increase in the electrolysis time from 10 to 60 min the yield of product increased. At higher electrolysis time of 60 min the number of nuclei formation at cathode surface was more. The size of particles was affected by electrolysis time. The increase in the particles size was observed with electrolysis time (Figure 4.2.6). So, electrolysis time is directly proportional to particle size (Goh et al., 2011).

4.2.4. Effect of Solvent

The effect of different solvents such as water, water-methanol and water-ACN on the size of Al₂O₃ NPs has been studied at 100 mA current and NaOH supporting electrolyte. In electrochemical reaction, the solution contains dissolved ions, which are mobile and support the current flow. The choice of good solvent in the electrochemical synthesis is another important factor. A solvent with good solvation power was required to dissolve the reactants and products. The low viscosity of solvent was needed for rapid transport of reactants and products to electrode. Solvent with low reactivity compatible with oxidizing and reducing electrodes and reactive species created at electrode. Aprotic solvents provide reduction and oxidation limits beyond the reused in aqueous solutions, so that a wider range of potentials became available for electrochemical studies. Acetonitrile a polar aprotic solvent, with high conductivity, solvating power, high dielectric constant ($\varepsilon = 37$) and nontoxicity has been used as solvent for the fabrication of metals and alloys (Seo et al., 2013). It has been observed that the reaction solution turned colorless to milky within few minute in water-ACN solvent as compared to water and water-methanol solvent. The yield of the product was recorded higher in water-ACN solvent compared to water-methanol
and water solvents. The change in shape and size of Al₂O₃ NPs in different solvents media was observed as shown in Figure 4.2.7.

The desired morphology, size and purity of Al₂O₃ NPs can be achieved by complete understanding of reaction mechanism of electrodeposition. On the basis of results, the possible mechanisms for generation of Al₂O₃ NPs in presence of NaOH, Na₂CO₃ and NaNO₃ electrolyte may be as follow:

**In presence of NaOH electrolyte**

Anode:

\[
\text{Al} \rightarrow \text{Al}^{3+} + 3e^-
\]

Cathode:

\[
\text{OH}^- + 2\text{H}_2\text{O} + 2e^- \rightarrow 3\text{OH}^- + \text{H}_2
\]

Electrolyte solution:

\[
2\text{Al}^{3+} + 6\text{OH}^- \rightarrow \gamma-\text{Al}_2\text{O}_3 + 3\text{H}_2\text{O} + \text{H}^+
\]

Calcination:

\[
\begin{align*}
\gamma-\text{Al}_2\text{O}_3 & \rightarrow \delta-\text{Al}_2\text{O}_3 & 900 ^\circ \text{C} & \rightarrow & 1100 ^\circ \text{C} & \rightarrow & 1200 ^\circ \text{C} & \rightarrow & \alpha-\text{Al}_2\text{O}_3 \\
\end{align*}
\]

**In presence of Na₂CO₃ electrolyte**

Anode:

\[
\text{Al} \rightarrow \text{Al}^{3+} + 3e^-
\]

Cathode:

\[
\text{CO}_3^{2-} + \text{H}_2\text{O} + e^- \rightarrow 2\text{OH}^- + \text{CO}_2
\]

Electrolyte solution:

\[
2\text{Al}^{3+} + 6\text{OH}^- \rightarrow \gamma-\text{Al}_2\text{O}_3 + 3\text{H}_2\text{O} + \text{H}^+
\]

Calcination:

\[
\begin{align*}
\gamma-\text{Al}_2\text{O}_3 & \rightarrow \delta-\text{Al}_2\text{O}_3 & 900 ^\circ \text{C} & \rightarrow & 1100 ^\circ \text{C} & \rightarrow & 1200 ^\circ \text{C} & \rightarrow & \alpha-\text{Al}_2\text{O}_3 \\
\end{align*}
\]

**In presence of NaNO₃ electrolyte**

Anode:

\[
\text{Al} \rightarrow \text{Al}^{3+} + 3e^-
\]
Cathode:

\[ \text{NO}_3^- + \text{H}_2\text{O} + 2\text{e}^- \rightarrow \text{OH}^- + \text{NO}_2^- \]

Electrolyte solution:

\[ 2\text{Al}^{3+} + 6\text{OH}^- \rightarrow \gamma-\text{Al}_2\text{O}_3 + 3\text{H}_2\text{O} + \text{H}^+ \]

Calcination:

\[ \begin{align*}
\gamma-\text{Al}_2\text{O}_3 & \rightarrow \delta-\text{Al}_2\text{O}_3 & \rightarrow \theta-\text{Al}_2\text{O}_3 & \rightarrow \alpha-\text{Al}_2\text{O}_3 \\
900 ^\circ \text{C} & \rightarrow 1100 ^\circ \text{C} & \rightarrow 1200 ^\circ \text{C}
\end{align*} \]

When current was passed through the electrolytic cell, OH\(^-\) and Al\(^{3+}\) ions are generated on the surface of cathode and anode. It was observed that Al\(^{3+}\) ions react with OH\(^-\) ions to produce Al\(_2\)O\(_3\) (Picard et al., 2000). Thus, the nature of electrolyte affects the shape and size of nanoparticles due to presence of different anions.

### 4.2.5. Characterization Techniques

#### 4.2.5.1. XRD Analysis

Figure 4.2.1 illustrates the XRD pattern of Al\(_2\)O\(_3\) NPs prepared in the presence of electrolyte SH, SC and SN with or without calcination. The broad and less intense diffracted lines were observed at 20 angles of 39°, 45°, 60° and 66° assigned to γ-Al\(_2\)O\(_3\) (Xu et al., 2009). The sample obtained after calcination at 900 °C and 1100 °C showed mixed phases of aluminum oxide (θ-Al\(_2\)O\(_3\) to α-Al\(_2\)O\(_3\)) (ICDD 29-0063) (ICDD 48-0366).

γ-Al\(_2\)O\(_3\) phase transforms to θ-Al\(_2\)O\(_3\) at 700-800 °C and θ-Al\(_2\)O\(_3\) phase metastable transforms to α phase at about 1050 °C. The phase transitions in aluminum oxide occurred due to order as follow (Liu et al., 2009; Rogojan et al., 2011; Gitzen et al., 1970).

\[ \begin{align*}
\gamma-\text{Al}_2\text{O}_3 & \rightarrow \delta-\text{Al}_2\text{O}_3 & \rightarrow \theta-\text{Al}_2\text{O}_3 & \rightarrow \alpha-\text{Al}_2\text{O}_3.
\end{align*} \]

Al\(_2\)O\(_3\) NPs obtained after calcination at 1200 °C, display peaks corresponding to α-Al\(_2\)O\(_3\) NPs (JCPDS No.46-1212). Similar pattern of diffraction peaks was observed in all electrolytes. The crystallinity of Al\(_2\)O\(_3\) NPs was increased as the calcination temperature increased from 900 °C to 1200 °C.
The peaks appeared at 2θ of 25.71º, 35.18º, 38.90º, 43.44º, 52.67º, 57.58º, 61.40º, 66.57º, 68.24º and 77.01º values corresponds to 012, 104, 110, 113, 024, 116, 018, 214, 300 and 119 lattice planes (Gangwara et al., 2011). The size of Al₂O₃ NPs was calculated from XRD data using Debye Scherrer equation. From the XRD data the average crystallite size were found to be 33 nm, 36 nm and 54 nm, respectively for SH, SC and SN electrolyte at 1200 ºC.
4.2.5.2. TEM Analysis

Figures 4.2.2-4.2.4 show TEM micrographs of Al$_2$O$_3$ NPs prepared at 100 mA current, water-ACN solvent and electrolyte (SH, SC and SN). TEM results inferred nanobars formation in presences of SH and SC supporting electrolyte with average grain size of 30 nm and 36 nm, respectively. Whereas, in presences of SN electrolyte spherical Al$_2$O$_3$ NPs with average grain size of 43 nm was formed. The selected area electron diffraction (SAED) pattern indicated the crystalline and ordered orientations of Al$_2$O$_3$ NPs (Figure 4.2.2b). The lattice plane spacing of 0.36 nm matches well with the lattice plane 012 of the α-AO NPs as inferred from Figure 4.2.3c and 4.2.4c. HRTEM images show the results compatible with the data obtained from the XRD analysis.

TEM results clearly showed the effects of current on the particle size of Al$_2$O$_3$ NPs as shown in Figure 4.2.5. The aluminum oxide prepared at 20 mA showed particles size 50-60 nm. At 50 mA current, particles size decreased to 30-50 nm. The results indicated that the particle size inversely proportional to the current.

The influence of electrolysis times on the size of particle are shown in Figure 4.2.6a-c. The incomplete formation of particles was observed within 10 min the electrolysis. It has been reported that the particles size increased with electrolysis time 30 min and average particle size of 25 nm was recorded 60 min electrolysis time. Increase in particles size with electrolysis time was clearly noticeable in the micrographs.

TEM micrograph revealed that the morphology and size of Al$_2$O$_3$ NPs was greatly influenced by solvents are shown in Figure 4.2.7. Al$_2$O$_3$ NPs prepared in water solvent showed two set of agglomeration. One set was small in size (average 20 nm) and other was large particle (average 140 nm) as shown in Figure 4.2.7a. TEM micrograph of Al$_2$O$_3$ NPs clearly indicated smaller particles formation in water-methanol solvent (20-30 nm) as compared to water-ACN solvent (30 nm). The results also confirmed that the morphology and size of nanoparticles changed with varying solvents.

4.2.5.3. UV-visible Analysis

UV-visible spectrum of Al$_2$O$_3$ NPs of nanoparticles in SH electrolyte, water-ACN solvent and 100mA current after calcined at 1200 °C is shown in Figure 4.2.8.
visible absorption spectroscopy of the aluminum oxide dispersed in distilled water showed an absorption peak around 210 nm. A strong absorption peak at 210 nm clearly confirmed the formation of Al$_2$O$_3$ NPs in deionized water (Piriyawong et al., 2012). The band gap energy of Al$_2$O$_3$ NPs was calculated using Tauc relation as ((Ray et al., 2001):

$$\alpha h\nu = \beta (h\nu - E_g)^n$$

where, \(\alpha\) = absorption coefficient = 2.303 A/l, 
E$_g$ = optical band gap, 
h\(\nu\) = photon energy, 
\(\beta\) = band tailing parameter, 
and \(n = \frac{1}{2}\) for direct band gap.

The optical band gap was determined by extrapolating the straight portion of curve between \((\alpha h\nu)^2\) and \(h\nu\) when, \(\alpha = 0\). The band gap as calculated from Tauc plot was found to be 3.2 eV as shown in Figure 4.2.8b (Kortov et al., 2008).

4.2.5.4. FTIR Analysis

FTIR spectrum of the Al$_2$O$_3$ NPs is shown in Figure 4.2.8c. The absorption band at 3467 cm$^{-1}$ was due to the O–H vibrations (Srivastava et al., 2011). The peaks at 574 cm$^{-1}$ and 826 cm$^{-1}$ was observed due to Al–O bonds (Rabiezadeh et al., 2012; Karim et al., 2011).

4.2.5.5. Thermal Analysis

Thermal analysis of Al$_2$O$_3$ NPs is shown in Figure 4.2.9. The total weight loss of 7.4\% was recorded at temperature between 100 °C and 600 °C. The initial decomposition temperature and final decomposition temperature was recorded as 93 °C and 341 °C. It may be due to the dehydration of adsorbed water (Sathyaseelan et al., 2013). DTA studies of Al$_2$O$_3$ NPs revealed only one exothermic peak at 58 °C due to decomposition stage in TGA at 93 to 341 °C.
Figure 4.2.2: TEM micrographs of Al₂O₃ NPs synthesized in presence of sodium hydroxide electrolyte (b) SADP pattern (c) HRTEM image (e) particle size count
Figure 4.2.3: TEM micrographs of Al₂O₃ NPs synthesized in presence of sodium carbonate electrolyte (b) SADP pattern (c) HRTEM image (e) particle size count
Figure 4.2.4: TEM micrographs of Al$_2$O$_3$ NPs synthesized in presence of sodium nitrate electrolyte (b) SADP pattern (c) HRTEM image (e) particle size count
Figure 4.2.5: TEM micrographs of Al₂O₃ NPs synthesized at (a) 20 mA (b) 50 mA current
Figure 4.2.6: TEM micrographs of Al₂O₃ NPs synthesized at different electrolysis time (a) 10 min (b) 30 min (c) 60 min
Figure 4.2.7: TEM micrographs of Al₂O₃ NPs synthesized in presence of (a) water solvent (b) water-methanol solvent (c) water-acetonitrile solvent
Figure 4.2.8: UV-Visible spectrum of Al$_2$O$_3$ NPs (b) plot of $(\alpha h\nu)^2$ vs $h\nu$ of Al$_2$O$_3$ NPs calcined at 1200°C (c) FTIR spectrum of Al$_2$O$_3$ NPs

Figure 4.2.9: TGA analysis of Al$_2$O$_3$ NPs
4.2.6. Applications of Aluminum oxide Nanoparticles (Al₂O₃ NPs)

Al₂O₃ NPs prepared in water-ACN solvent, SH electrolyte, 100 mA current at 1200 °C has been selectived for photocatalytic and antimicrobial activity.

4.2.6.1. Photocatalytic Degradation of Dyes

Photocatalytic degradation of different dyes such as methylene blue (MB), congo red (CR) and methyl red (MR) has been studied using nanoparticles. The photocatalytic activity of Al₂O₃ NPs for the degradation of MB, CR and MR dye in UV–visible irradiation was recorded for 120 min.

The decreases in absorbance band intensities in presence of Al₂O₃ NPs with irradiation time were observed for MB, MR and CR as indicated in Figure 4.2.10. The direct photolysis of MB, CR and MR dye under UV–visible light irradiation is negligible, suggesting that the dye is chemically stable. The adsorption capacity of dyes without Al₂O₃ NPs was about 2-3 % in 120 min as shown in Figure 4.2.10b. The absorbance decreased gradually with exposure time for all dyes in presence of Al₂O₃ NPs. The degradation of 40 %, 90 % and 16 % was recorded for MB, CR and MR dyes, respectively within 120 min of irradiation. The degradation of congo red was observed higher as compared to other dye.

Figure 4.2.10d-e shows the plot of ln A₀/A vs irradiation time for MB and CR. The linear correlation with good precision was observed. This indicated the pseudo-first-order rate order kinetics for dyes degradation. The rate constant of 0.01318 min⁻¹ and 0.10679 min⁻¹ were recorded for MB and CR. Figure 4.2.10f shows the plot of ln 1/A vs irradiation time and compared the pseudo-second-order rate order kinetics for MR dye degradation. The rate constant (2.55437 m⁻¹⋅c⁻¹) for MR was recorded. The mechanism for catalytic degradation of dyes was already discussed in section 4.1.6.1.

4.2.6.2. Antimicrobial Activity of Al₂O₃ NPs

The antimicrobial activity of Al₂O₃ NPs was studied against E. coli, S. aureus, A. nigres and C. albicans strain using growth curve method. The results showed very less growth inhibition in all the cases. No significant inhibition of Al₂O₃ NPs was observed against E. coli, S. aureus, A. nigres and C. albicans strain.
Figure 4.2.10: (a) Photodegradation of MB, CR and MR with Al₂O₃NPs (b) without Al₂O₃NPs under UV-light (c) % of degradation of dyes (d) plot of ln $A_0/A_t$ versus time for MB (e) CR and (f) plot of $1/A$ versus time for MR dye
### Table 4.2.1. Preparation of Al₂O₃ NPs at different experimental conditions

<table>
<thead>
<tr>
<th>Reaction parameters</th>
<th>Solvents</th>
<th>Electrolytes</th>
<th>Current</th>
<th>Reaction time</th>
<th>Stirring rate (r/s)</th>
<th>TEM (Average particle size (nm))</th>
</tr>
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<tr>
<td>Effect of electrolyte</td>
<td>Water-ACN</td>
<td>NaOH</td>
<td>100 mA</td>
<td>2 h</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>water-ACN</td>
<td>NaNO₃</td>
<td>100 mA</td>
<td>2 h</td>
<td>5</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Water-ACN</td>
<td>Na₂CO₃</td>
<td>100 mA</td>
<td>2 h</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td>Effect of solvent</td>
<td>Water</td>
<td>NaOH</td>
<td>100 mA</td>
<td>2 h</td>
<td>5</td>
<td>20, 140 (two set of particles)</td>
</tr>
<tr>
<td></td>
<td>Water-methanol</td>
<td>NaOH</td>
<td>100 mA</td>
<td>2 h</td>
<td>5</td>
<td>20-30</td>
</tr>
<tr>
<td></td>
<td>Water-ACN</td>
<td>NaOH</td>
<td>100 mA</td>
<td>2 h</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>Effect of current</td>
<td>Water-ACN</td>
<td>NaOH</td>
<td>20 mA</td>
<td>2 h</td>
<td>5</td>
<td>50-60</td>
</tr>
<tr>
<td></td>
<td>Water-ACN</td>
<td>NaOH</td>
<td>50 mA</td>
<td>2 h</td>
<td>5</td>
<td>30-50</td>
</tr>
<tr>
<td>Effect of electrolysis time</td>
<td>Water-ACN</td>
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<td>10 min</td>
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<td>Incomplete formation</td>
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<td></td>
<td>Water-ACN</td>
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<td>100 mA</td>
<td>30 min</td>
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<td>Not countable</td>
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<td></td>
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<td>NaOH</td>
<td>100 mA</td>
<td>60 min</td>
<td>5</td>
<td>25</td>
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</table>
4.3. Synthesis of Guar gum/copper oxide Nanocomposites

Several samples of CuO NPs have been prepared electrochemically by varying reaction parameters such as electrolyte, solvent, current and electrolysis time. CuO NPs prepared in presence of sodium hydroxide, water-ACN for 120 min at 100 mA and calcined at 900 ºC having particle size 20 nm has been selected for the composite studies. The GG/CuO nanocomposite was formed due to binding of organic part guar gum and inorganic part (CuO NPs) which can be explained through the mechanism as shown in Scheme 4.3.1.

GG/CuO nanocomposite was explored for different applications such as photocatalysis, antimicrobial activity and cytotoxicity study. GG/CuO nanocomposite has been characterized by different techniques. These are discussed as follow:

4.3.1. Characterization Techniques

4.3.1.1. XRD Analysis

Figure 4.3.1 shows the XRD pattern of GG/CuO nanocomposite. XRD results confirmed the strongest peaks at (111) (202) indicated the preferential growth directions. The diffraction peaks at 32.5º, 35.4º, 38.7º, 48.7º, 53.5º, 58.3º, 61.5º, 67.9º, 72.4º and 75.0º corresponds to the (110), (1 1 1), (111), (202), (020), (2 2 0), (113), (311), (220) and (311) lattice planes of the CuO NPs (Zhu et al., 2013). The strong and sharp reflection peaks inferred the crystalline nature of the nanocomposite. The broadening of peaks suggested the size of particle in nanoscale. The pure guar gum shows characteristic peaks at 20º which corresponds to peak of GG (Mine et al., 2010). The mean size of the particle calculated from Scherrer’s equation based on XRD data was about 25 nm

\[
D = \frac{0.9\lambda}{\beta \cos \theta}
\]

where \(\beta\) is full width half maxima (FWHM) of the diffraction peak, \(\theta\) is the Bragg peak angle and \(\lambda\) is the wavelength of Cu K\(\alpha\) radiation.
Step-1 Electrochemical synthesis of CuO NPs

Anode: \( \text{Cu} \rightarrow \text{Cu}^{2+} + 2\text{e}^- \)

Cathode: \( \text{OH}^- + 2\text{H}_2\text{O}^+ + 2\text{e}^- \rightarrow 3\text{OH}^- + \text{H}_2 \)

In electrolytic solution: \( \text{Cu}^{2+} + \text{OH}^- \rightarrow \text{CuO} + \text{Cu}_2\text{O} \)

Calcined at 900°C

\( \text{CuO} \)

Step-2 Synthesis of GG/CuO Nanocomposite

\[ + \text{CuO} \quad \text{K}_2\text{S}_2\text{O}_8 \quad 50^\circ\text{C} \]

GG/CuO Nanocomposite

Scheme 4.3.1: Schematic Illustration for the Synthesis of GG/CuO Nanocomposite
4.3.1.2. FTIR Analysis

FTIR spectrum of GG and GG/CuO nanocomposite are shown in Figure 4.3.2. The characteristic band at 586 cm\(^{-1}\) and 525 cm\(^{-1}\) were observed due to Cu-O starching vibration. In case of GG, a broad band at 3381 cm\(^{-1}\) and 2927 cm\(^{-1}\) assigned to O-H stretching vibration and C-H stretching of CH\(_2\) group, respectively.

![Figure 4.3.1: XRD pattern GG/CuO Nanocomposite](image)

The band at 1445 cm\(^{-1}\) and 1076 cm\(^{-1}\) corresponds to C-H and O-H bending. A sharp band at 1650 cm\(^{-1}\) appeared due to ring stretching of galactose and mannose. Two bands at 1155 cm\(^{-1}\) and 1022 cm\(^{-1}\) may be due to C-O-C and C-O stretching (Wang et al., 2009). The band at 871 cm\(^{-1}\) was due to C-H deformation mode (Singh et al., 2009). The change in the intensity of some function groups and appearance of new peaks in the spectrum further confirmed the formation of nanocomposite.

4.3.1.3. TEM Analysis

TEM micrograph of GG/CuO nanocomposite showed monodispersed nanoparticles are shown in Figure 4.3.3. TEM micrograph of GG/CuO nanocomposite inferred the formation of spherical particles size between 20 nm and 50 nm.
Figure 4.3.2: FTIR spectra of GG and GG/CuO nanocomposite

Figure 4.3.3: TEM image of GG/CuO nanocomposite
4.3.1.4. SEM Analysis

Scanning electron microscopy (SEM) micrographs of GG/CuO nanocomposite and pure GG are shown in Figure 4.3.4a - b. SEM micrographs revealed that CuO NPs immobilized onto guar gum. It indicated the binding of the organic polymer with inorganic material changed the morphology of composite material.

![SEM images](image)

Figure 4.3.4: SEM images of (a) guar gum (b) GG/CuO Nanocomposite

4.3.1.5. UV-visible Spectroscopy Analysis

UV-visible spectrum and band gap studies of GG/CuO nanocomposite are shown in Figure 4.3.5a-b. The absorption peak at 300 nm was recorded for GG/CuO nanocomposite (Yan et al., 2012). The band gap of GG/CuO was calculated using Tauc relation. The optical band gap is determined by extrapolating the straight portion of curve between $(\alpha h\nu)^2$ and $h\nu$ when, $\alpha = 0$. The band gap as calculated from Tauc plot was found to be 1.8 eV is shown in Figure 4.3.5b.
Figure 4.3.5: (a) UV-visible absorption spectrum of GG/CuO nanocomposite and (b) band gap spectra of GG/CuO nanocomposite

4.3.1.6. Thermo Gravimetric Analysis

Figure 4.3.6 shows the thermo gravimetric curve of guar gum. It is evident that 3.7 % weight loss was observed at 89 °C due to dehydration. Thermal degradation of GG was completed in two steps, 251 °C to 308 °C and 308 °C to 518 °C. First stage of decomposition was observed with maximum weight loss of 47.5 % between 251 and 308 °C due to the breakdown of conjugation bond. After 308 °C weight loss was very less which increased further with temperature. Only 40.3 % weight loss was observed in the temperature between 308 °C to 518 °C. In DTA studies, GG have exhibited one exothermic peak at 291 °C in the temperature range from 251 °C to 308 °C in TGA spectra. Figure 4.3.7 shows the TGA curve of GG/CuO nanocomposite. In case of GG/CuO nanocomposite, degradation occurred due to loss of water molecule upto temperature of 32 °C with weight loss of 0.2 %. The three decomposition stages have been observed for GG/CuO nanocomposite. First stage decomposition temperature was occurred from 32 to 186 °C with weight loss of 0.4%. Second stage of decomposition temperature was from 186 to 320 °C with weight loss of 54 % and third decomposition temperature was occurred from 320 to 567 °C with 1.1 % weight loss.
Total weight loss upto final decomposition temperature was recorded to be 6.5 %. GG/CuO nanocomposite was found thermally more stable than the raw material. In DTA studies GG/CuO nanocomposite showed three exothermic peaks at 49 °C, 224
°C and 268 °C which assigned to decomposition stage between 32-186 °C, 186-320 °C and 320-567 °C, respectively in TGA.

4.3.2. Applications of Guar gum/copper oxide Nanocomposite (GG/CuO)

4.3.2.1. Photocatalytic Degradation of Dye

The degradation of MG dye removal using GG/CuO nanocomposite was studied under two reaction conditions i.e. equilibrium adsorption in dark followed by photocatalysis and synergistic adsorption/photocatalysis directly in sunlight irradiation. The results of photocatalytic degradation of MG dye are shown in Figure 4.3.8.

1\textsuperscript{st} reaction condition: equilibrium adsorption in dark followed by photocatalysis.

GG in nanocomposite acted as adsorbent for the removal dye from water system. The adsorption–desorption equilibrium of MG dye was established in dark. During equilibrium adsorption the composite was highly covered by dye molecules which prevent the sunlight and suppress the further degradation of dye (Xu et al., 2001). It has been observed that only 32% of MG dye was removed by GG/CuO under dark conditions within 60 min. Further photocatalysis of adsorbed and free dye present in solution, the degradation of about 87% was recorded after irradiation of 120 min as shown in Figure 4.3.8c and Table 4.3.1. During irradiated electron–hole pairs are generated, which produce hydroxyl and super-oxide radicals which interrupt the conjugation in organic molecules. UV– visible spectral changes of MG with reaction time under in equilibrium adsorption followed by photocatalysis are shown in Figure 4.3.8a. The mechanism of equilibrium adsorption followed by photocatalysis is presented as follow (Gupta et al., 2015):

**Step-1 (in dark at equilibrium)**

GG/CuO + Dye (Free in solution) → GG/CuO-Dye (adsorbed)
GG/CuO-Dye (adsorbed) → GG/CuO + Dye (Free in solution)

**Step–II followed by photocatalysis in sunlight**

GG/CuO-Dye (adsorbed) and Dye (Free in solution) + hv → GG/CuO (e\textsuperscript{−} + h\textsuperscript{+}) Dye

\[ h^+ + H_2O \rightarrow OH^- + H^+ \]

\[ h^+ + OH^- \rightarrow OH^- \]
e− + O2 → O2•−

O2•− or OH’ + Dye (adsorbed and Free in solution) → Intermediate Product → Degraded Product.

The plot of ln Co/Ct vs irradiation time shown a linear correlation was shown in Figure 4.3.9e GG/CuO nanocomposite followed pseudo-first-order kinetics for the photodegradation of MG dye (Pathania et al., 2014). Thus adsorption followed by photocatalysis of dyes was fitted to pseudo-first-order kinetics (Rupa et al., 2007). The value of rate constant k and correlation coefficient (R^2) for MG are given in Table 4.3.1.

2nd reaction condition: synergistic effect of adsorption/photocatalysis directly in sunlight

The photocatalytic degradation of MG dye by GG/CuO nanocomposite was found out to be 89% in 180 min as shown in Figure 4.3.8d. It has been revealed that the degradation of MG dye proceeded faster. The synergistic effect of adsorption/photocatalysis of MG dye involved the simultaneous adsorption of dye onto photocatalyst and generation of electron hole pair from catalyst (Julkapli et al., 2014). The degraded dye products left the surface of GG/CuO nanocomposite free for further adsorption and photocatalysis. UV–visible spectral changes of MG with reaction time under visible light in synergistic adsorption/photocatalysis are shown in Figure 4.3.8b. The proposed mechanism for the synergistic adsorption/photocatalysis degradation of dye was shown as follows:

GG/CuO + MG Dye → GG/CuO-Dye adsorbed (Sunlight)
GG/CuO (h+) -Dye adsorbed + H2O → GG/CuO (OH’)-Dye + H+ 
GG/CuO (h+) -Dye adsorbed + OH’ → GG/CuO (OH’)-Dye
GG/CuO (e−) -Dye adsorbed + O2 → GG/CuO (O2•−)-Dye
O•−2 or OH’ + Dye- GG/CuO →Intermediate Product →Degraded Product + Free GG/CuO for reuse.

In case of synergistic process the adsorption of dyes onto the photocatalyst surface and generation of electron-hole pairs occurs at same time. The free radicals disrupt the conjugation which can promote mineralization of dye (Siriwong et al., 2012).
Figure 4.3.8: Spectrum of MG (a) adsorption in dark followed by photocatalysis (b) synergistic adsorption/photocatalysis. (c) adsorption in dark followed by photocatalysis, % degradation of MG. (d) synergistic adsorption/photocatalysis, % degradation of MG. (e) adsorption in dark followed by photocatalysis, pseudo-first-order kinetics for photodegradation of MG. (f) synergistic adsorption/photocatalysis, pseudo first order kinetics for photodegradation of MG.
Decomposition of conjugated structure, N-demethylation reactions, hydroxyl addition reactions, removal of benzene ring, and ring-opening reaction mechanism of MG was reported in literature (Yong et al., 2015). Photocatalytic efficiency can be enhanced by adsorption of adsorbate onto the catalyst. The adsorption of dye on to adsorbent can facilitate the photodegradation process and increased the degradation rate and also reduces the degradation time. As adsorbed dye molecules were more prone to attack of radicals than the dissolved dye. The kinetics of MG dye degradation shows a linear correlation as shown in Figure 4.3.8f. Thus synergistic adsorption/photodegradation of dyes by GG/CuO nanocomposite was fitted to pseudo-first-order kinetics (Yong et al., 2015). The value of rate constant and regression coefficients ($R^2$) value for MG are given in Table 4.3.1.

4.3.2.2. Antibacterial Activity of GG/CuO Nanocomposite

The antibacterial activity of GG/CuO nanocomposite was tested against S. aureus. It has been observed that the GG/CuO nanocomposite inhibits the growth of S. aureus bacteria, through binding with the outer membrane of S. aureus. The antibacterial effect was recorded more pronounced at high concentration of nanocomposite as revealed from Figure 4.3.9. The antibacterial effect of nanocomposite may be due to the inhibition of dehydrogenase enzyme, periplasmic enzyme activity and active transport (Mansour et al., 2014).

4.3.2.3. Cytotoxicity Studies of GG/CuO Nanocomposite

Figure 4.3.10 shows the cytotoxicity of GG/CuO nanocomposite assessed on three cell lines, viz. CHO-K1, KB and C6 using different concentrations after 24, 48 and 72 hours exposition. Maximum cytotoxicity of GG/CuO nanocomposite was observed with C6 cell line at concentration of 25 μg/ml (~24 %) at 72 hours and ~20% at 48 h. After 24 h no significant cytotoxicity was observed in any of the three cell lines. From the cytotoxicity data, it was concluded that no cytotoxicity effect of the GG/CuO nanocomposite for CHO-K1 cells, oral cancer cells KB and rat glioma C6 cells over a wide range of temperature, concentration and exposition time.
Figure 4.3.9: Antibacterial efficiency of GG/CuO nanocomposite against *S. aureus* bacteria

<table>
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<th></th>
<th>0 hour</th>
<th>24 hours</th>
<th>48 hours</th>
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<tbody>
<tr>
<td>Control (<em>S. aureus</em>)</td>
<td>450000000</td>
<td>2E+17</td>
<td>1.35E+19</td>
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<tr>
<td>Composite (0.4 µg/ml)</td>
<td>380000000</td>
<td>9E+12</td>
<td>2.1E+16</td>
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<tr>
<td>Composite (0.8 µg/ml)</td>
<td>420000000</td>
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<td>1.8E+14</td>
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</table>

Figure 4.3.10: Cytotoxicity patterns of GG/CuO nanocomposite on three cell lines, viz. CHO-K1, KB and C6
Table 4.3.1. % Removal of MG by GG/CuO nanocomposite under sunlight, rate constants and linear coefficients from log $C_0/C_t$ vs T plots for MG

<table>
<thead>
<tr>
<th>Dye</th>
<th>Concentration</th>
<th>Adsorption followed by photocatalysis</th>
<th>Synergetic effect of adsorption/photocatalysis</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>% degradation in (60 min)</td>
<td>% degradation in (180 min)</td>
</tr>
<tr>
<td>MG</td>
<td>1.5 x 10$^{-5}$</td>
<td>32</td>
<td>87</td>
</tr>
</tbody>
</table>
4.4. Synthesis of Guar gum/aluminum oxide Nanocomposites

Different sample of aluminum oxide nanoparticles have been synthesized by varying the reaction parameters such as electrolyte, solvent, current and electrolysis time. It was inferred that the aluminum oxide prepared in presence of sodium hydroxide electrolyte, water-ACN for 120 min at 100 mA and calcined at 1200 ºC was selected for the fabrication of composite. The nanocomposite of GG/Al₂O₃ was formed due to the binding of organic part (guar gum) and inorganic part (Al₂O₃ NPs) (Scheme 4.4.1)

GG/Al₂O₃ nanocomposite was explored for different applications such as photocatalysis, antimicrobial activity and cytotoxicity study. GG/Al₂O₃ nanocomposite has been characterized by different techniques. These are discussed as follow:

4.4.1. Characterization Techniques

4.4.1.1. XRD Analysis

XRD pattern of GG/Al₂O₃ nanocomposite is shown in Figure 4.41. XRD result shows the crystalline nature of nanocomposites. XRD exhibits the characterization peaks of aluminum oxide (Al₂O₃) at scattering angles of 2θ of 35.5º, 32.9º, 38.1º, 61.3º, 67.1º. All the peaks of Al₂O₃ were compared to JCPDS No.46-1212 standard file (Gangwara et al., 2011). The broadening of peaks indicated the formation of GG/Al₂O₃ nanocomposite.

4.4.1.2. FTIR Analysis

FTIR analysis of GG and GG/Al₂O₃ nanocomposite are shown in Figure 4.4.2. For GG spectra, the peaks at 3600-3400 cm⁻¹ were assigned to O-H stretching vibrations of water molecule. The absorbance peaks at 2927 cm⁻¹ and 1445 cm⁻¹ corresponds to C-H stretching and C-O-C asymmetric bending (Yan et al., 2012). The peaks at 1155 cm⁻¹, 1023 cm⁻¹ and 1676 cm⁻¹ may be due to C-O-C stretching, hydroxylic C-O single band stretching of C-O-C group in the anhydroglucose ring and ring stretching of galactose and mannose band, respectively (Khan et al., 2013). The change in the intensity of some function groups and appearance of new peaks in the GG/Al₂O₃ spectrum also confirmed the formation of nanocomposite. The sharp peaks at 533 cm⁻¹, 553 cm⁻¹ corresponds to Al-O-Al bending mode. A weak peak at 821 cm⁻¹ may be due to Al-O stretching vibration (Kim et al., 2009).
Step-1 Electrochemical synthesis of Al₂O₃ NPs

Anode: \[ \text{Al} \rightarrow \text{Al}^{3+} + 3\text{e}^- \]

Cathode: \[ \text{OH}^- + 2\text{H}_2\text{O}^+ + 2\text{e}^- \rightarrow \text{3OH}^- + \text{H}_2 \]

In electrolytic solution: \[ 2\text{Al}^{3+} + 6\text{OH}^- \rightarrow \gamma\text{-Al}_2\text{O}_3 + 3\text{H}_2\text{O} + \text{H}^+ \]

Calcination: \[ \gamma\text{-Al}_2\text{O}_3 \xrightarrow{500^\circ \text{C}} \delta\text{-Al}_2\text{O}_3 \xrightarrow{1100^\circ \text{C}} \theta\text{-Al}_2\text{O}_3 \xrightarrow{1200^\circ \text{C}} \alpha\text{-Al}_2\text{O}_3 \]

Step-2 Synthesis of GG/Al₂O₃ Nanocomposite

Scheme 4.4.1: Schematic illustration of the synthesis process of GG/Al₂O₃ nanocomposite
4.4.1.3. TEM Analysis

TEM micrographs of Al₂O₃ NPs and GG/Al₂O₃ nanocomposites are shown in Figure 4.4.3. TEM micrographs inferred the dispersed homogeneous particles with diameters between 20 nm and 45 nm. Micrograph clearly inferred that the light part guar gum wrapped dark part of Al₂O₃ NPs and particles were quite well dispersed on guar gum.

4.4.1.4. SEM Analysis

Figure 4.4.4a-b illustrates the SEM micrographs of guar gum and GG/Al₂O₃ nanocomposites. The guar gum exhibits bean shape morphology. SEM micrograph of GG/Al₂O₃ shows the agglomeration of particles and formation of the nanocomposites.

![Figure 4.4.1: XRD pattern of GG/Al₂O₃ nanocomposite](image)

4.4.1.5. UV-visible Analysis

Figure 4.4.5 shows the UV-visible spectra of GG/Al₂O₃ nanocomposite. The absorption band at about 210 nm attributed due to Al₂O₃ NPs (Chang et al., 2008; Kortov et al., 2004) and band shifting at 240 nm were evident for GG/Al₂O₃ nanocomposite.
Figure 4.4.2: FTIR spectra of GG and GG/Al₂O₃ nanocomposite

Figure 4.4.3: TEM micrographs of GG/Al₂O₃ nanocomposite
The absorption peak of GG/Al$_2$O$_3$ nanocomposites showed evident red-shift phenomenon which proved the strong interactions between polymer matrix and Al$_2$O$_3$ NPs. The shifting of the absorption bands was due to the interaction between a polymer matrix and nanoparticles (Gangwara et al., 2011). The band gap of composite was calculated using Tauc relation.

The optical band gap was determined by extrapolating the straight portion of curve between $(\alpha h\nu)^2$ and $h\nu$ when, $\alpha = 0$. The band gap as calculated from Tauc plot was found to be 3.02 eV.

4.4.1.6. Thermogravimetric Analysis

Thermogravimetric curves of GG/Al$_2$O$_3$ nanocomposites are shown in Figure 4.4.6. The weight loss at temperature from 30°C to 585°C was found to be 11.8%. The decomposition of nanocomposite occurred in three stages. In first stage 5.8% weight loss occurred at decomposition temperature of 100 °C, which confirms the loss of hydrogen bonded water molecule present at the surface. Second and third stage decomposition were recorded from 200 to 310 °C and 310 to 517 °C with weight loss 4.6 and 1.4 %, respectively. It may be due to degradation of organic part. It is evident from the TGA data that GG/Al$_2$O$_3$ nanocomposites were thermally more stable. In DTA studies GG/Al$_2$O$_3$ nanocomposites three exothermic peaks at 47 °C, 290 °C and 502 °C assigned to decomposition stage between 31- 100°C, 200- 310 °C and 310- 517 °C, respectively in TGA.
Figure 4.4.5: UV-Visible spectra of (a) GG/Al$_2$O$_3$ nanocomposite and (b) plot of $(ahv)^2$ vs $hv$

Figure 4.4.6: TGA graph of GG/Al$_2$O$_3$ nanocomposite
4.4.2 Applications of Guar gum/aluminum oxide Nanocomposites (GG/Al₂O₃)

4.4.2.1. Photodegradation of dye

Photocatalysis of GG/Al₂O₃ nanocomposites was studied at different processes such as adsorption in dark followed by photocatalysis and synergistic adsorption/photocatalysis under sunlight irradiation as shown in Figure 4.4.7. For the adsorption followed photocatalysis process, the MG dye solution containing GG/Al₂O₃ nanocomposites in dark established the adsorption/desorption equilibrium.

For further photodegradation process the solution was exposed to sunlight for the adsorption of free dye present in solution. The decrease in absorption band intensities in presence of nanocomposite with irradiation time was observed for MG. It clearly confirmed that the dye was degraded effectively by nanocomposite (Figure 4.4.7a). It has been observed that only 41% of MG dye was adsorbed by GG/Al₂O₃ in dark conditions and 76% of MG dyes degraded in sunlight irradiation under adsorption followed photocatalysis process at 180 min (Figure 4.4.7c). The surface of photocatalyst is covered by dye molecules and degradation is suppressed. But when solution was irradiated under sunlight, electron hole pair is generated, which reacts with water to release hydroxyl and super oxide radical (Kumar et al., 2014). The super oxides break the conjugation of molecules. Adsorption followed by photocatalysis the mechanism was earlier reported by our research group (Gupta et al., 2015). A plot of ln A₀/Aᵣ vs irradiation time showed a linear correlation for GG/Al₂O₃ nanocomposites (Figure 4.4.7e). Thus adsorption followed by photocatalysis of dyes was fitted to pseudo-first-order kinetics (Kumar et al., 2014). The rate constant k and correlation coefficient (R²) for MG were shown in Table 4.4.1.

The synergistic adsorption/photocatalysis process directly in sunlight involves the adsorption of dye onto the material and photodegradation of dyes molecules at the same time. UV–visible spectral changes of MG in synergistic adsorption/photocatalysis are shown in Figure 4.4.7b. The photocatalytic degradation percentage
of MG dyes by GG/Al$_2$O$_3$ nanocomposites was found to be 87 % in 180 min of photoirradiation as shown in Figure 4.4.7d. The higher removal of dye was achieved as compared to adsorption followed by photocatalysis. In synergistic process, we assumed simultaneous adsorption of dyes onto the photocatalyst surface and generation of electron-hole pairs on absorption of light at same time and generate free radicals, to disrupt the conjugation in the adsorbed and free dye molecules present in the solution (Ueda et al., 2008). The degraded dye products left the surface of nanocomposite free for further adsorption and photocatalysis. The dye adsorption onto adsorbent can facilitate the photodegradation process and increased the degradation rate and reduces the degradation time (Gupta et al., 2015). The proposed mechanism for the synergistic adsorption/photocatalysis degradation of dye are shown as follows:

$$\text{GG/Al}_2\text{O}_3 + \text{MG Dye} \rightarrow \text{GG/Al}_2\text{O}_3 - \text{Dye adsorbed (Sunlight)}$$

$$\text{GG/Al}_2\text{O}_3 (h^+) - \text{Dye adsorbed} + \text{H}_2\text{O} \rightarrow \text{GG/Al}_2\text{O}_3 (\text{OH}^-) - \text{Dye} + \text{H}^+$$

$$\text{GG/Al}_2\text{O}_3 (h^+) - \text{Dye adsorbed} + \text{OH}^- \rightarrow \text{GG/Al}_2\text{O}_3 (\text{OH}^-) - \text{Dye}$$

$$\text{GG/Al}_2\text{O}_3 (e^-) - \text{Dye adsorbed} + \text{O}_2 \rightarrow \text{GG/Al}_2\text{O}_3 (\text{O}_2^-) - \text{Dye}$$

$$\text{O}_2^+ \text{ or } \text{OH}^- + \text{Dye- GG/Al}_2\text{O}_3 \rightarrow \text{Intermediate Product} \rightarrow \text{Degraded Product} \rightarrow \text{Free GG/Al}_2\text{O}_3 \text{ for reuse.}$$

Thus photodegradation was better in synergistic adsorption/photocatalysis condition. The kinetics of MG dye degradation shows a linear correlation as shown in Figure 4.4.7f. Thus, synergistic adsorption/photodegradation of dyes was fitted to pseudo-first-order kinetics.

The value of rate constant and regression coefficients ($R^2$) for MG are given in Table 4.4.1. The comparison of rate constants for synergistic adsorption/photocatalysis and adsorption followed photocatalysis clearly indicates that the photocatalysis under synergistic conditions was more efficient and effective. The exceptional
photodegradation performance of nanocomposite enhances its properties for waste water treatment.

4.4.2.2. Antibacterial Activity

The antibacterial activity of prepared GG/Al₂O₃ nanocomposite was screened against S. aureus. It is evident that GG/Al₂O₃ nanocomposite inhibited the growth of S. aurus bacteria. It was due to the binding of nanocomposite with the outer membrane of cell wall and preventing the nutrient transport inside the cell, dehydrogenase enzyme, periplasmic enzyme activity and active transport. The antibacterial effect was recorded more pronounced at high concentration of nanocomposite (Figure 4.4.8) (Mansour et al., 2014).

4.4.2.3. Cytotoxicity Studies of GG/Al₂O₃ Nanocomposites

Fig. 4.4.9 shows the cytotoxicity of GG/Al₂O₃ nanocomposites evaluated on three cell lines, viz. CHO-K1, KB and C6. As observed, after 24 h no cytotoxicity was observed at any of the cell line. Maximum cytotoxicity achieved by GG/Al₂O₃ nanocomposites at 48 h was ~21% at maximum concentration of the sample (100μg/ml) with KB cell line. KB showed maximum cytotoxicity ~30% again at maximum concentration at 72 h. No significant cytotoxicity of nanocomposite was observed with CHO-K1 and C6 over a wide range of temperature, concentration and exposition time.
Figure 4.4.7: Photodegradation spectrum of MG (a) adsorption in dark followed by photocatalysis, (b) synergistic adsorption/photocatalysis. (c) adsorption in dark followed by photocatalysis, % degradation of MG. (d) synergistic adsorption/photocatalysis, % degradation of MG. (e) adsorption in dark followed by photocatalysis, pseudo-first-order kinetics (f) synergistic adsorption/photocatalysis, pseudo first order kinetics
Chapter 4

Results and Discussion

Figure 4.4.8: Antibacterial efficiency of GG/Al₂O₃ nanocomposite against S. aureus bacteria

Figure 4.4.9: Cytotoxicity patterns of GG/Al₂O₃ nanocomposite on three cell lines, on three cell lines, viz. CHO-K1, KB and C6
Table 4.4.1. % Removal of MG by GG/Al₂O₃ nanocomposite under sunlight, rate constants and linear coefficients from log C₀/Cₜ vs T plots for MG

<table>
<thead>
<tr>
<th>Dye</th>
<th>Concentration</th>
<th>Adsorption followed by photocatalysis</th>
<th>Synergetic effect of adsorption/photocatalysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% degradation in dark</td>
<td>% degradation in light</td>
</tr>
<tr>
<td>MG</td>
<td>1.5 x 10⁻⁵</td>
<td>41</td>
<td>81</td>
</tr>
</tbody>
</table>