



Copper Oxide Microspheres for Pseudocapacitor Applications and its Cytotoxicity Studies

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The preparation of copper oxide (CuO) microspheres was done via hydrothermal route with the amphiphilic polymer pluronic (P123) as the capping agent. The XRD studies confirmed the monoclinic structure of the compound. The formation of microspheres were confirmed through SEM and TEM studies. The pseudo-capacitance properties were inferred with three electrode system. The cyclic voltammetry studies were done for the scan rates of 5 to 100 mV/Sec. The charge-discharge analysis was done for different current density values of 1 to 20 A/g. The specific capacitance of the material for a current density value of 1 A/g was found to be 245 F/g. The cytotoxicity study of the CuO microspheres studied using MTT assay shows the material to be non-toxic in nature.

Keywords: Copper oxide, Microspheres, Hydrothermal route, Pseudocapacitor, Cytotoxicity

1 Introduction

Supercapacitors with high energy and power density are considered to be the bridge between the batteries and capacitors, due to which it has gained the attention of researchers¹. But, the practical energy densities achieved by supercapacitors are too low to meet the demand for hybrid electric vehicles², and this could be improved using the pseudo capacitor materials. The materials like Ruthenium Oxide (RuO₂) and Manganese Oxide (MnO₂) are the well explored materials for pseudo capacitors. Copper oxide (CuO), a crucial p-type metal oxide semiconductor is an interesting material with potential applications in photocatalysis, sensors, solar driven water splitting, *etc*³⁻⁵. If we look in terms of its economic and environmental view points, the CuO is an excellent electrode material for energy applications by its low cost, low toxicity, and abundance in nature. A recent report in 2018 clearly shows the copper oxide (CuO/Cu₂O) as one of the promising metal oxides for pseudo capacitor applications, among the other metal oxides like RuO₂, MnO₂, V₂O₅, SnO₂, Fe₂O₃, Co₃O₄, and *etc*⁶. Though various research has been carried out with this interesting material for supercapacitors,

the nanotoxicology study report of Cu/CuO nanoparticles shows it to be both cytotoxic and genotoxic in nature⁷. Any material used in energy applications is supposed to be non-cytotoxic in nature, for the sustainability of living organisms. Hence the focus of our research is to prepare CuO microspheres with simple method and to study its pseudo capacitor performance and as well as its cytotoxic nature. The properties of the CuO material is morphology dependent. The CuO with microsphere morphology was reported for its better properties⁸ and applications like ethanol sensing⁹⁻¹⁰, glucose sensing¹¹, and lithium ion battery¹². The exploration of CuO microspheres for supercapacitor application is not yet reported. Our work on CuO microspheres by hydrothermal method has shown the material to exhibit a specific capacitance of 245 F/g for a current density of 1 A/g, indicating it as a best pseudocapacitor material. The cytotoxicity of the CuO microspheres in our present study shows the material to be very safe for the environment.

2 Experimental methods

2.1 Materials for preparing Copper Oxide

The tri block copolymer Pluronic (P123) made of hydrophylic poly(ethylene oxide) (PEO) groups and hydrophobic poly(propylene oxide) (PPO) group in an

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alternating linear fashion, *i.e.* PEO-PPO-PEO [PEO₂₀PPO₇₀PEO₂₀] was used as the surfactant. It has a molecular weight of, $M_w = 5800$ g/mol and was obtained in high purity from Himedia. The copper acetate of 99.9 %purity (Sigma Aldrich) was used as the copper source.

2.2 Preparation of Copper Oxide

The 2.32 g of the surfactant Pluronic 123 was added to the ethanol and water mixture which was taken in 50:50 ratio. It was kept for stirring at room temperature for about 2-3 hours. After the homogenous mixture was attained, the copper acetate was added to the above stirring solution and allowed for another 2-3 hours of stirring after which a light blue solution was obtained. To the above light blue solution, ammonia was added till slightly darker blue color was observed and the pH turns to 7. Later it was taken to be kept in the hydrothermal setup at 180-200 °C for 12 hrs. After cooling naturally, the black coloured precipitate formed was collected. To remove the unwanted residues, it was centrifuged thrice with water and ethanol after which it was dried for 12 hrs at 80 °C. The material was then taken as such for further characterizations.

2.3 Characterization methods

The X-ray Diffraction (XRD) pattern of the as prepared materials was obtained using the instrument SHIMADZU-XRD-6000 diffractometer (Cu-K_α radiation of wavelength $\lambda = 0.1541$ nm) as the source at 40 kV. The morphology studies of the as prepared material were done with the instrument JEOL-JSM 6000, Scanning Electron Microscope (SEM). The Transmission Electron Microscopy (TEM) and the Selected Area Electron Diffraction (SAED) studies were done using JEOL-JEM 2100. The electrochemical properties of the as prepared sample were measured with the electrochemical workstation (CH Instruments, CHI600E). The three electrode working system was used to measure the electrochemical performance of the material under study. The platinum wire was used as the counter electrode, and the saturated calomel electrode (SCE) was utilised as the reference electrode. The 3M of potassium hydroxide (KOH) was used as the aqueous electrolyte solution. The cyclic voltammetry (CV) of the three electrode system was carried out at scan rates of 5 to 100 mV/Sec. The galvanostatic charge-discharge (GCD) tests were made at the current density values ranging from 1-20 A/g.

2.4 Experimental details on cytotoxicity studies

Since the exposure of metal oxides particularly in nano size is hazardous by contact through skin and inhalation¹³, the cytotoxic effect of as prepared CuO microspheres becomes essential. In the present work, the copper oxide microspheres were tested in the cancer cell by the cell viability test. The terazolium salt (MTT) is the most common method for the assessments of the cell viability, where the yellow colored dye get reduced and becomes an insoluble crystalline blue product called as Formazan by the cellular enzymes. A calorimeter is used to determine the cell viability. This is due to the presence of cellular enzyme called as the NAD(P)H-dependent oxidoreductases. The formazan crystals are later dissolved using appropriate solubilizing solution and the solution appears dark, if there are more metabolically active cells. Later, the absorbance is measured in the 500-600 nm range using the plate reader.

2.5 Preparation of Reagents

The MTT stock solution was prepared by magnetically stirring 500 mg of MTT powder in the 10 ml of phosphate buffer solution in a dark room. Later a 0.22 mm filter (Millipore, Ireland) was used to filter the sterilized solution, and stored in 10 ml aliquots (50 mg/ml) at a temperature of -20 °C. The 5 mg/ml of the diluted solution was then taken as the working solution made on the day of the experiment.

The cell lines also called as the vero cells are used for the assessment of the toxins, and chemical substances in mammalian cells at the molecular level. For the present research work, the vero cell lines were purchased from the National Cancer Center of Science, Pune, India. The 5% Fetal bovine serum was purchased from the Sigma Aldrich, USA. The cell lines were then cultured and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), glutamine (2 raM), 100 units/ml of penicillin and 100 lg/ml of streptomycin. Later the cells were cultured in a 5% humidified carbon dioxide incubator at a temperature of 37 °C.

2.6 Protocol

The cells allowed to adhere for about twenty four hours at a temperature of 37 °C in the carbon dioxide incubator in a 96 well flat bottom micro titer plate with 1×10^4 cells/well. Later, the cell medium was replaced for a fresh one. To this, 10 μ L of the MTT working solution prepared as discussed above was added to each well and incubated for four hours. The

formazan crystals formed were dissolved in the solvent DMSO for thirty minutes, where the amount of formazan crystals was quantified with ELISA plate reader.

3 Results and discussion

3.1 X-ray Diffraction analysis

X-ray diffraction analysis was used to determine the crystalline phase of the as prepared material. The Fig. 1 shows the XRD pattern of the material to have well pronounced diffraction peaks at 2θ values of 32.1, 35.3, 38.2, 48.4, 53.4, 57.8, 61.4, 66.2 & 74.6 ° corresponding to the planes (110), (002), (111), (-202), (020), (202), (-113), (311) and (222) hkl reflection planes, respectively of monoclinic phase of copper oxide¹⁴. The observed peaks were found to agree with the JCPDS Card no. 48-1548. No other impurity peaks were found to occur. The average crystallite size of CuO microspheres were found to be 10.3 nm.

3.2 Morphology analysis

The SEM images of the copper oxide particles are shown in Fig. 2(a-c) at different magnifications. The SEM images made at lower magnification shows the formation of copper oxide microspheres of different diameters (Fig. 2a). The higher magnification (Fig. 2b) shows a single microsphere which is sphere shaped. Further increase in magnification (Fig. 2c) clearly shows the CuO microsphere particles to be of ~1.8 μm in size and in turn made of numerous nanoparticles attracted to each other to form the microsphere. This clearly shows the role of the surfactant pluronic in the formation of microspheres,

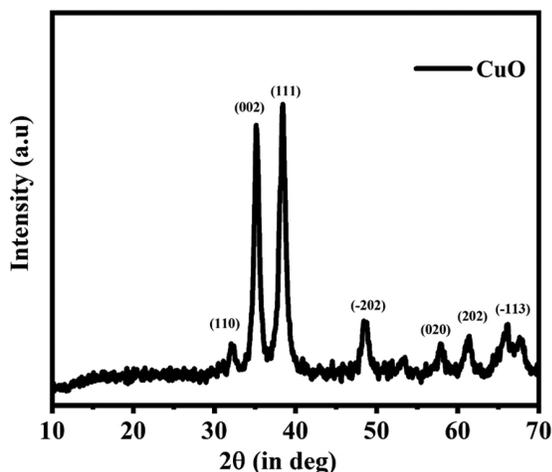


Fig. 1 — XRD pattern of copper oxide

where the micelles of hydrophilic and hydrophobic groups are formed in the solvent medium (Fig. 3). While the hydrophilic PEO group interacts with the water medium, the hydrophobic PPO group interacts with the ethanol. When copper acetate is added to the above micelle media, the copper ions get capped by the surfactant (Fig. 3). The micelles also help to disperse the copper ions in the solvent medium and act as the nucleation center for the growth of copper oxide microspheres. The hydrothermal treatment of the above mixture for about 12 hrs gives enough energy for the formation of copper oxide, while the particles get tuned to form microspheres by the capping of surfactant. The TEM analysis (Fig. 4a), shows the formation of perfect microsphere shaped CuO particles and were found to be in particle size range of 800 nm to 1.2 μm . The SAED pattern (Fig. 4b) shows the copper oxide microspheres to be polycrystalline in nature, as also confirmed from the XRD analysis, as discussed above. The SAED pattern

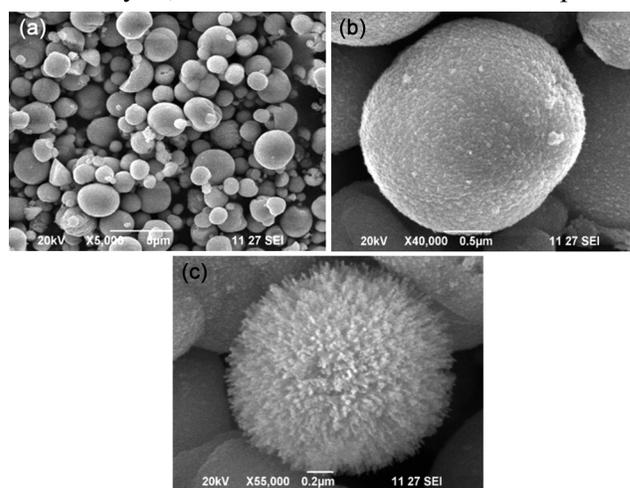


Fig. 2 — (a-c) SEM images of copper oxide microspheres at different magnification

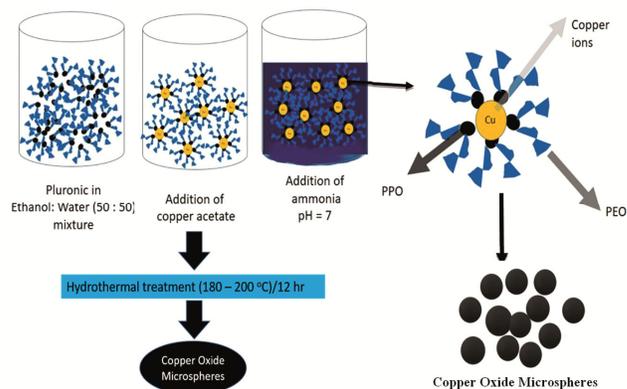


Fig. 3 — Schematic diagram for formation of micelles

reflected the (111) (hkl) plane with a d-spacing of 2.354 Å.

3.3 Electrochemical Studies

The CV studies of the material recorded using the three electrode system as mentioned under the experimental part, is shown in Fig. 5(a). As the scan rate increases, the current value was found to increase, indicating the capacitive nature¹⁵. The appearance of redox peaks indicate the pseudocapacitive nature of the copper oxide microspheres. While the oxidation happens during the anodic process, the reduction happens during the cathodic process. The oxidation peaks appeared at 0.22 V during the anodic process at a scan rate of 5 mV/sec was found to slightly keep

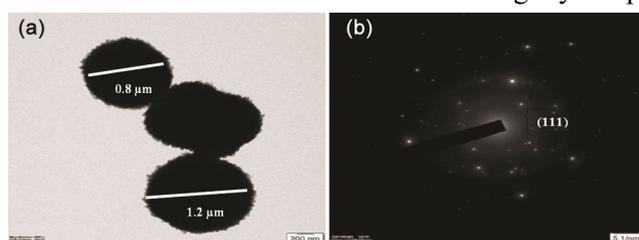


Fig. 4 — (a) TEM image of copper oxide microspheres and its (b) SAED pattern

shifting to 0.3 V, for a scan rate of 100 mV/sec. Similarly, the cathodic peak appearing at 0.15 V for a scan rate of 5 mV/sec shifted to 0.9 V for a scan rate of 100 mV/sec. The degree of capacitive nature of the CuO microspheres was found by knowing the slope value 'b' of the log (peak current) vs. log (sweep rate) plot. By the power law¹⁶, the relationship between the peak current and the scan rate is given as,

$$I_p = k v^b \quad \dots(1)$$

$$\log I_p = b \log v + \log k \quad \dots(2)$$

Where ' I_p ' is the peak current (in Ampere) and ' v ' is the potential scan rate (in V/sec). The ' k ' and ' b ' are the arbitrary coefficients. If the ' b ' value is 0.5, the material is considered to be diffusion limited, where the charge storage happens by the ion diffusion. With the slope value of 1, the material is considered to be capacitive in nature, where the charge storage happens by surface adsorption/desorption process. As shown in the Fig. 5(b), the graph was plotted as per Eq (1) and (2), and the slope value of $b = 0.61$ and $b = 0.65$ was calculated using

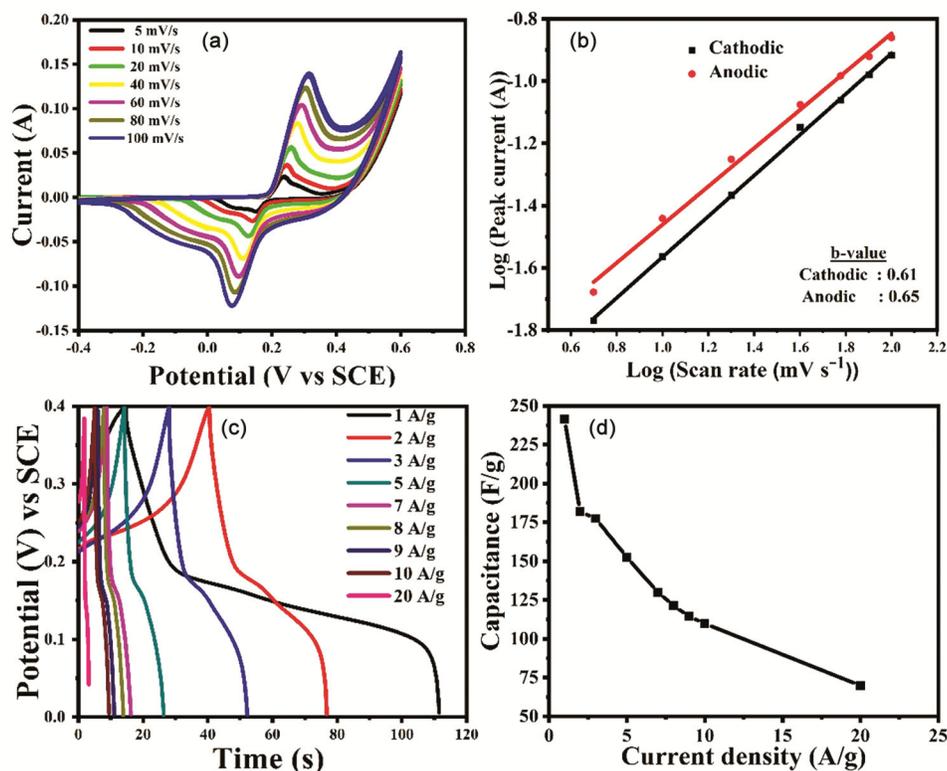


Fig. 5 — (a) Cyclic Voltammetry at the potential window of -0.4 to 0.6 V at a scan rate of 5 to 100 mV/sec, (b) log (peak current) vs. log (scan rate) and (c) GCD studies in the potential window of 0 to 0.4 V and (d) the first cycle specific capacitance for different current densities of 1 to 10 A/g

the cathodic and anodic peak currents, respectively. These values indicate the CuO microspheres to show pseudocapacitive nature, which is a combination of capacitive and insertion process, and similar property of CuO was reported in its thin film form¹⁶. The galvanostatic charge-discharge studies in the current density range of 1 - 20 A/g is shown in the Fig. 5(c). The shape of the discharge curve shows the pseudocapacitive effect, which would be otherwise a triangle shaped in nature for a pure capacitor. The value of the specific discharge capacitance was calculated using the formula¹⁷,

$$C = \frac{I\Delta t}{m\Delta V} \quad \dots(3)$$

where 'C', 'I', 'Δt', 'm' and 'ΔV' are the capacitance in units of F/g, current in units of ampere, time difference in units of seconds, electrode active mass in units of grams and potential difference in units of volts, respectively. The specific capacitance calculated using the above formula was found to be 245 F/g for the current density of 1 A/g. With increase in the current density, the specific capacitance decreases due to the slow kinetics of the ion at the high current. Still the CuO microsphere delivered a reasonable specific capacitance of more than 100 F/g even for a high current density of 10 A/g.

3.4 Cytotoxicity Studies

The cytotoxicity is calculated by subtracting the value of absorbance reading of the blank from all the samples (Fig. 6). The percentage of cell viability (% CV) was calculated using the Eq. 4. If the absorbance is greater than the control, then it indicates the cell proliferation. If it is lower value, then it suggests the cell death or it indicates the inhibition of the proliferation.

$$\% CV = \left(\frac{\text{mean OD}}{\text{control OD}} \right) * 100 \quad \dots(4)$$

The dose-response curve of the cell viability (in %) vs the sample concentration (in μg/ml) was plotted (Fig. 7), from which the cytotoxicity concentration that kills 50 % of the Vero cells (IC₅₀) was obtained. The most widely used Half-maximal inhibitory concentration (IC₅₀) is an informative measure of a drug's efficacy. It indicates the amount of the drug needed to inhibit the biological process by half.

As explained in detail, the cytotoxic potential of the copper oxide was studied using MTT assay

(Figs. 6-7 & Table. 1). The assay is based on the principle that the viable cells will contain the enzyme called the mitochondrial dehydrogenase that converts the dye into blue coloured formazan dye. The absorbance produced is directly proportional to the number of viable cells. The IC₅₀ value is the concentration at which the sample exhibits 50% inhibition of growth of cells. Lower the IC₅₀ value higher the toxicity. The sample showed the IC₅₀ values (434.53) higher than the chemotherapeutic standard drug called the 5-fluorouracil values (134 μg/ml) which is toxic to Vero cell lines¹⁸. It suggests that the samples are non-toxic to normal cells.

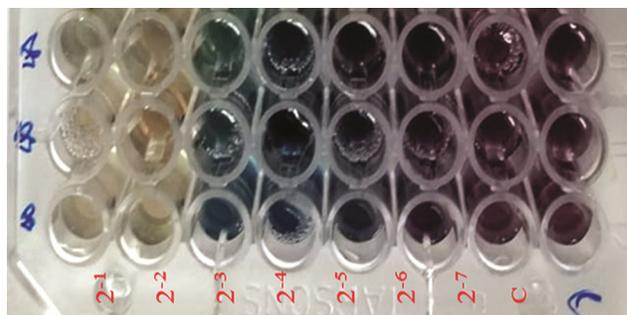


Fig. 6 — Cytotoxic assay of copper oxide microspheres

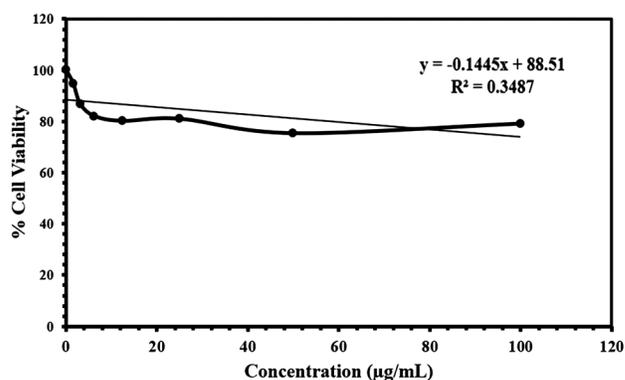


Fig. 7 — Dose response curve of Vero cell lines following treatment with copper oxide microspheres

Table 1 — Cytotoxic effect of Sample against Vero cell line

| Material | Concentration (μg/ml) | Mean OD at 570 nm | % Cell Viability | IC ₅₀ |
|---------------------------|-----------------------|-------------------|------------------|------------------|
| | 0 | 0.521 | 100.0961 | |
| | 1.562 | 0.493667 | 94.8447 | |
| | 3.124 | 0.451667 | 86.77554 | |
| | 6.25 | 0.427 | 82.0365 | |
| Copper oxide microspheres | 12.5 | 0.417333 | 80.17931 | 434.53 |
| | 25 | 0.421667 | 81.01185 | |
| | 50 | 0.392333 | 75.37624 | |
| | 100 | 0.411667 | 79.09062 | |

4 Conclusion

Copper Oxide microspheres were prepared by the surfactant assisted hydrothermal method. The formation of micelles by the surfactant pluronic in the water medium at suitable temperature acted as a capping agent for the controlled growth of microspheres. Phase purity of the copper oxide microspheres and its surface morphology were confirmed using XRD and SEM analysis, respectively. Electrochemical analysis showed the suitability of the material to be used in supercapacitors. The CV studies show the material to behave as a pseudocapacitor with well-defined redox peaks at all the scan rates. The specific capacitances were found to be 245 and 100 F/g for a current density value of 1 and 10 A/g, respectively. The non-cytotoxicity nature of copper oxide microspheres as tested using MTT assay shows the material to be more favourable for the environment and hence can be utilised for the energy applications.

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