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Fabrication and characterization of a colorimetric sensor using EDTA-capped copper oxide nanoparticles for phenolic compound detection

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ABSTRACT

The catalytic and antibacterial properties of metal oxide nanoparticles make them suitable contributors to the formation of new production facilities at low cost. In this study, we prepared copper oxide nanoparticles (CuONPs) via an environmentally friendly and cost-effective synthesis route. We used Ethylene diamine tetra acetic acid (EDTA) as a capping and reducing agent to synthesize copper oxide nanoparticles. Textural characterization of the prepared CuONPs was studied using advanced techniques, for example, Fourier transmission infrared (FTIR) to study the functional transition (EDTA reduction), scanning electron microscopy (SEM) to analyze the shape of CuONPs, X-ray diffraction patterns to examine the crystalline nature of the particles. CuONPs were then used to perform amperometry measurements and monitor trace amounts of isoflavone (IFs) in various industrial wastewater and phytoestrogen drugs from patients and water samples. The results show that the developed sensor has excellent analytical parameters such as high sensitivity as a sensor, uniformly distributed, controlled size, high stability at neutral pH, good selectivity (no interference), and a cost-effective method for preparing and fabricating mid-frequency sensors. In this research oxidation state and particle size of nanoparticles played a vital role in enhanced catalytic process. EDTA-CuONPs with the smallest particle size, biocompatibility, surface reactivity, uniform distribution and assembled morphology, and enhanced catalytic activity make them ideal for applications including environment monitoring, catalysis, and biosensing.

1. Introduction

An innovative field of science that we know as nanoscience and nanotechnology. It is based on the size of the materials in the range of 1-100 nm with different textural features such as shapes and dimensions of the materials like as nano spherical, nanorods or nanofibers, nanobelts, nanoribbons, 2-d, nanosheets like as graphene oxide, Silica oxide sheets, 3-d nanostructures, and others have much more interest in the field of research [1]. At the nanoscale size, the characteristics of the material have completely changed as compared with micro bulk-sized materials [2]. The properties, like physical and chemical, also change due to it possessing a greater surface-to-volume ratio; it enhances the material's efficiency as well as sensitivity more than a thousand times [3]. There are many studies reported that nanomaterials can be prepared with different low-cost methods including chemical, physical as well as biological (green methods), and so on [2, 4, 5]. Nowadays, nanomaterials are widely available and highly useful in the daily routine of life applications,

like medical diagnostics, fabrications, drug delivery process, nano colloidal medicine, pharmaceuticals, nano cosmetics for beauty, nanofibers, treatment of water as well as other purification processes and also widely used as a semiconductor in the fabrication of the electronic devices [6, 7]. Furthermore, metal oxide nanomaterials have been intensively used for energy conversion applications such as solar cells, fuel cells, and photo voltaic cells, and catalytic applications possess much more attention in new fields of research such as electro-catalysts as well as photo-catalysts [8, 9].

Metallic nanoparticles have all three dimensions within the 1 to 100 nm range. Nowadays, metallic nanoparticles have gained wide attention because of their shape and size-dependent properties [8, 10]. Gold, silver, and other metal nanoparticles have a strong history since 6000 BC. These particles have been used for various rituals in ancient times, and metal nanoparticles were used in ornaments, food, drinks, and treatment purposes [11]. The characteristic behavior of metal nanoparticles is studied by their size and shape. on the other hand, the strong and most interesting side of metal nanoparticles is their optical properties, which depend on the size and shape of the particles [12]. Metal nanoparticles have a large number of bonding sites, possess the ability to store a large number of electrons, and have unique and specific chemical properties because of this [13, 14]. Metal nanoparticles have wonderful applications in sensing and imaging, such applications are also because of their optical properties [15, 16]. Different methods, sciences, and technologies are working on metal nanoparticles. Scientists and researchers are using these particles for several purposes, including human health and environmental issues [17]. These metal nanoparticles are helpful and could be applied for water purification; it is also observed that these particles have wonderful antibacterial properties. Not only this, but they also have magnetic properties that have long-term applications [18, 19]. Many compounds could be used for the synthesis and stability of these particles to make them more applicable and unique [20], there are different methods to synthesize metal nanoparticles including the chemical reduction method which is a reliable, economical, and less time-consuming method, metal nanoparticles could form with the help of plants as well [10, 21, 22]. These particles have gained attention due to their friendly approach and can be easily characterized by different techniques [23].

Metal oxide nanostructures in which copper oxide nanostructures exhibit outstanding chemical, optical, and electric properties; belong to the monoclinic crystal system [24-26]. CuONPs show distinctive optical properties and intense color change due to interaction with phenolic compounds, which is the fundamental principle behind using it as a calorimetric sensor [27]. The surface plasmon resonance properties of copper are stronger in UV-visible regions. CuONPs are known for their superior chemical stability and resistance to oxidation, which is an excellent property to use as a sensor. They can resist numerous pH and temperature conditions. Also, these particles can be targeted to detect specific analytes. For this reason, CuO nanoparticles are used widely than other materials, like gold or silver nanoparticles, which may oxidize under some conditions. CuONPs are found to be highly electric as well as thermally conductive, have photovoltaic features, have antimicrobial activity, are relatively stable for long periods, and have cost-effective procedures for preparation as well as modification [28-30]. Recently, applications of CuONPs are the most important to exhibit electrochemical water-splitting properties [31]. There are several methods for the preparation of CuONSs, which have different morphology as well as textural topography with different sizes [32, 33]. In previous work, researchers have developed methods for the preparation of CuONPs, wet chemical, biological (green), sonochemical, and solvothermal such as hydrothermal or alcohol-thermal growth route, indirect or direct thermal decomposition, colloid–synthetic process, microwave-assisted radiation growth process, quick-precipitation and also electrochemical synthetic procedure [24].

In this study, CuONPs were prepared using a moderate-temperature hydrothermal growth process. This method is cheaper as well as environmentally friendly because we have not used any hazardous chemicals during the preparation of CuONPs. However, such a synthesis method has not been reported using EDTA to synthesize CuONPs as a sensor.

2. Material and Methods

2.1 Materials and Methods

All analytically pure reagents were used in this work, and solutions were prepared in MilliQ water. Salts and chemicals were purchased from Merck (Germany), such as copper chloride dihydrate CuCl2.2H2O (99%), potassium hydroxide, KOH (98%), sodium hydroxide (NaOH, 97%), methionine (C5H11NO2S) 99%. To prepare buffer solutions we used dihydrogen phosphate (NaH2PO4), monohydrate phosphate (Na2HPO4), and potassium chloride salts. The drug Phytoestrogen was purchased from a local pharmacy and was used without further purification, the drug

was prepared in solvent. The capping agent EDTA and Copper were purified and used for further preparation using the standard synthesis method. Prepare a 1% Nafion solution using isopropyl alcohol. We followed all standard protocols to prepare real samples for applications and detection. For this purpose, solvents and other reagents were also used in purified conditions without further purification. UV-visible spectrometer, Fourier Transform Infrared Spectrometer, and other characterization techniques are used to understand the physical and chemical properties of CuONPs.

2.2 Synthesis of EDTA-CuONPs

The green approach was performed to prepare the EDTA-CuONPs in a tightly capped vessel. In this synthetic procedure, a green plant named Parthenium plant was extracted and spiked in a volumetric flask, and 150µL of 1mM standard solution of CuCl2.H2O was added in a 10 mL test tube after that 300ul of Ethylene diamine tetra acetic acid (reducing agent, capping agent, and stabilizing agent) was added in the same tube. Furthermore, 400ul of ultrapure milli Q water was added for dilution and was pipetted from 0.2 mM solution of NaOH. The CuONP formation was increased using NaOH because it acted as a catalyst and reduction agent. Finally, the diluted solution was stirred with the vertex mixture. The formation of CuONPs was observed with the help of a transparent solution mixture slowly changing into a deep brown color within 12 minutes. The pH of the solution mixtures was monitored close to the neutral range to attain nanoparticles of small size and shape. Physically, colorimetric techniques (change in color) confirmed the synthesis of CuONPs. Finally, prepared EDTA-CuONPs were used for the colorimetric detection of Isoflavone (IFs) and Phytoestrogens.

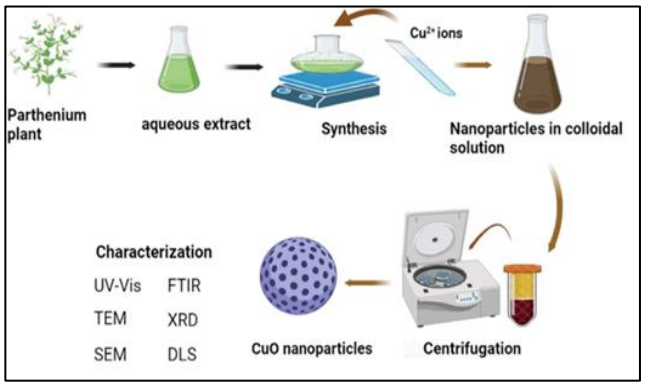

Fig. 1. Synthesis Process of EDTA-CuONPs

2.3 The colorimetric detection of IFs and Phytoestrogens

In this study, we developed a colorimetric sensor for monitoring the IFs and Phytoestrogen in the aqueous environment at normal temperature.

Phenolic compounds have a hydroxyl (OH) group, which acts as a reducing agent. Hence, when phenol reacts with CuONPs it reduces Cu (II) (oxidized copper) on the surface of the nanoparticles to Cu (I) or even Cu (0) (elemental copper). This reduction changes the optical properties and electronic structure of CuONPs making them eligible for further applications. Experimentally, different volumes of 1 mM standard solution IFs were added in the 200-4 ml with 200ul volume of EDTA-CuONPs added in the same solution. Then, this solution mixture was diluted to reach 5mL. The solution mixtures were taken for 2-5 minutes, and 3 mL of each sample was transferred into a 1 cm cuvette for the UV-spectral analysis. The absorbance of the blank solution was examined in the region at a wavelength from 200 to 800 nm without analyte. The visual response was shown in the variation of color from reddish brown to green via colorimetric analysis. The images of color change were taken with a camera after 5 minutes of reaction time. Similarly, the colorimetric detection process was repeated for Phytoestrogen detection under UV study. However, this color change depends upon the size and shape of synthesized nanoparticles, concentration, and type of phenolic compounds. As we used IFs and Phytoestrogen, IFs color changed to light green, and for Phytoestrogen the color changed to light purple.

2.4 Characterization Techniques

Characterization is the main aspect of understanding and evaluating the functional, physical, and chemical properties of EDTA-CuONPs. Hence, we followed different characterization techniques to understand these properties, such as UV-visible spectroscopy, X-ray diffraction technique (XRD), Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Zeta Potential, and Atomic Force Microscopy. These analytical techniques helped to understand morphology, size, shape, resonance, visible region, structure, chemical bonds, and other chemical and physical parameters.

2.5 Preparation of samples for XRD, AFM, FTIR, TEM, and SEM Analysis

A solution of EDTA-assisted CuONPs was prepared by heating 50 ml of EDTA-CuONPs in a 50 ml beaker on a hot plate, Temperature was maintained up to 55 degrees Celsius, until the solution reached 10 ml. Then the 10 ml solution was poured on a Petrie dish and heated at 55oC until dried. In 50 minutes, a black dry powder-like product was obtained, and centrifuged 5 times under 60 rpm and no heat. After centrifugation, the product was washed three times to remove in case of any impurities left, unreacted antibiotics, and unwanted substances. Washing was

carried out with water and ethanol in a separatory funnel. The product was heated in the oven for 2 hours under 55 degrees Celsius when dried it was our EDTA-CuONPs ready for characterization, hence these nanoparticles were collected in a pin drop for further analysis.

Fig. 2. Sample EDTA-CuONPs in a Petrie Dish After Heat

And In Powder Form Used For Characterization

2.6 Collection and Preparation of Samples

Real water and blood samples were collected from 10 different amenities, including industrial and medical zones and hospitals. water samples were used as collected, but the blood samples were centrifuged for 20 minutes at 2500 rpm and then stored in test tubes for application purposes. A few microliters of EDTA-CuONPs solutions were spiked into collected water and stored blood samples to study any changes in pH or UV visibility. Also, the EDTA-CuONPs solution was spiked with different concentrations of the IFs and Phytoestrogen to observe any physical or chemical changes. To be sure we performed a pH and UV Visible study, and it was observed that our synthesized EDTA-CuONPs can detect Ifs and Phytoestrogens from real samples.

3. Results and Discussion

3.1.SEM Analysis

Many advanced techniques have been used to investigate the sizes as well as shapes of the non-material including scanning electron microscope (SEM), Atomic force microscope (AFM), and X-ray diffraction (XRD). As-synthesized EDTA-CuONPs have been characterized with the mentioned techniques, likewise, the size and structure of the NPs have been investigated with the use of SEM, and we found 7 to 28 nm-sized nano-circles that fall within the size of CuONPs in previously reported methods, that is (Amin et al., 2017 Nithya et al. 2021). In addition, SEM was used to examine the circle-like shape of the prepared CuONPs. These round structures show less agglomeration and pure synthesis of EDTA-CuONPs; it also suggests that the nanoparticles are synthesized with high purity. This is in contrast with the reported method by (Zhang, H., et al. 2017).

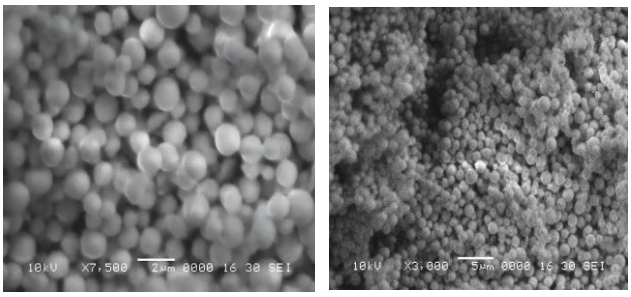

Fig. 3. SEM Images of EDTA-CuONPs Showing Nanostructures

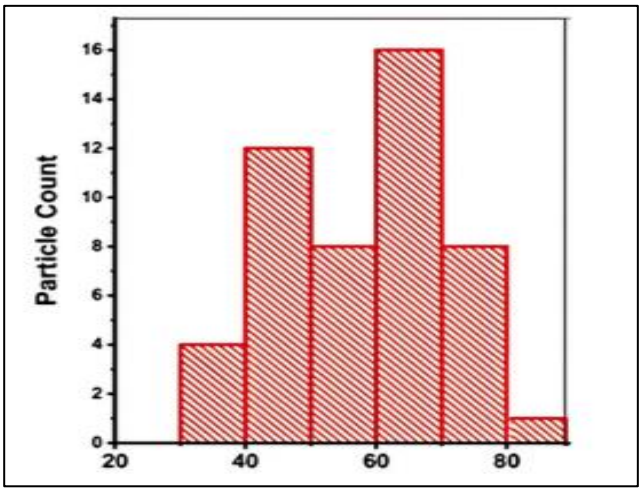

Fig. 4. SEM Size Distribution Graph of EDTA-CuONPs

3.2. AFM images of EDTA-CuONPs

AFM provided insights into the nanoparticles' size, surface, and aggregation and supported the SEM findings. The grain-like structure is clearly shown in AFM, and the range is between 0 and 100 nm. EDTA CuO Nanoparticles are uniformly distributed, clearly show a grain-like structure, and are well dispersed throughout the surface. This dispersion is important for sensor application because it influences sensor sensitivity. This finding supports another research where copper oxide nanoparticles were synthesized by the green synthesis method (S. S. Alqahtani et al., 2023). Also, compared to other conventional methods for synthesizing copper nanoparticles, which results in larger and poorly dispersed particles, our synthesis method for EDTA provides a more controlled and uniform dispersion and particle size. Our findings are the latest and unique from different reported studies.

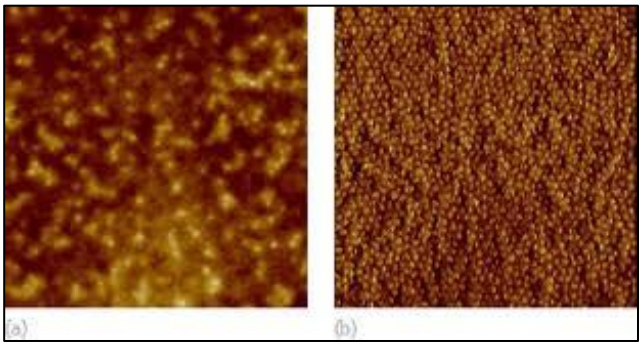

Fig. 5. AFM Images of EDTA-CuONPs

3.3. Stability and formation of EDTA-CuONPs

UV-visible and pH studies were conducted to evaluate the formation and stability of EDTA-CuONPs. The peak shift in absorbance peaks using different concentrations of plant extract confirms the synthesis of copper oxide nanoparticles in the visible region. However, peak shift is also observed due to the interaction of copper ions with plant components. This peak shift suggests that 2 mM plant extract helps reduce copper ions to CuO nanoparticles.

Our pH study indicates that the EDTAP-CuONPs are stable at a neutral pH. However, neutral pH is essential for applications of CuONPs as sensors, also reported in (N. M. S. Garcia et al., 2023). However, room temperature works for the sensor's stability, and this sensor could be stored at 18 degrees Celsius to avoid any contamination for several days. Our study's neutral pH and its stability are also advantageous because they will increase the chances of more advanced applications in environmental, vitro, vivo, and aqueous conditions, where there are more chances of pH fluctuation.

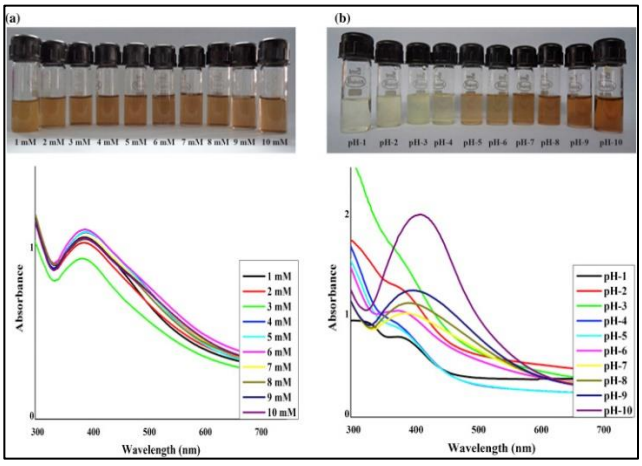

Fig. 6. pH Evaluation and UV-vis Spectra of EDTA-CuONPs

Fig. a shows different concentrations of parthenium plant extract in EDTA-CuONPs resulting in peak shifts at different ranges, confirming the synthesis of nanoparticles in the visible region with a 2mM solution of extract added.

Fig. b shows the pH of synthesized EDTA-CuONPs resulting in peak shift at different ranges. However, a neutral pH was maintained.

3.4 X-ray diffraction Analysis

XRD analysis of synthesized EDTA-CuONPs confirms the existence of three prominent peaks at 2 theta values of 210, 200, and 120 respectively. This finding confirms the crystal nature of EDTA-CuONPs with an average size of around 28.5 nm. The average size of the Nps was calculated using the Scherrer equation by the above XRD data. This value is confirmed by a previously reported method where CuONPs were synthesized by the green method (Bukhari et al., 2022). The crystal nature of our synthesized nanoparticles is important for the optical, catalytic, and electrical properties of nanoparticles, which are crucial for sensor performance. In our findings by XRD, we can achieve such a crystal nature because sharp diffraction peaks in XRD confirm the high crystal nature of EDTA-CuONPs, we can expect to achieve enhanced electronic and optical properties for future applications of these nanoparticles.

Moreover, lattice constant parameters and d spacing values were calculated by Bragg's law which were found to be 0.28 and 0.19 nm, these values are also comparable with previously reported green synthesis methods of CuONPs. These parameters can provide additional information about the growth of particles and how it affects the sensor's response time and sensitivity, recently such information was also mentioned in the paper (S. S. Alqahtani et al., 2024).

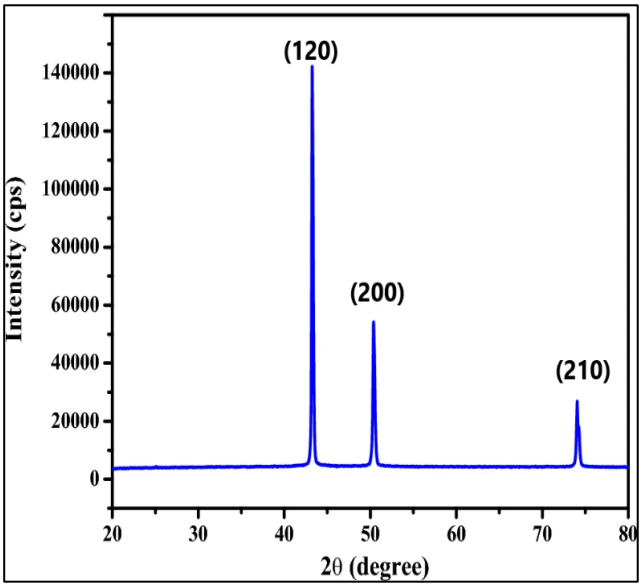

Fig. 7. XRD Diffraction patterns of EDTA-CuONPs

3.5 FTIR Study

Fourier Transform Infrared Spectroscopy (FTIR) helped to examine the functional groups associated with EDTA, Plant extract, and EDTA-CuONPs. FTIR spectra show the functional group of plant extract in the range of 4000 to 400 cm-1. OH, the functional group's peak at 1600 cm-1 is visible, which confirms the role of plant extract as a reducing and capping agent of the CuO nanoparticles. This supports a previous study that phenolic compounds in plant extracts stabilize nanoparticles and reduce agglomeration (X. Zhang et al., 2023). The formation of EDTA-CuONPs could also be confirmed from the peaks 3200 cm-1, 1330 cm-1, 1665 cm-1, 820 cm-1, 1020 cm-1, and 1230 cm-1.

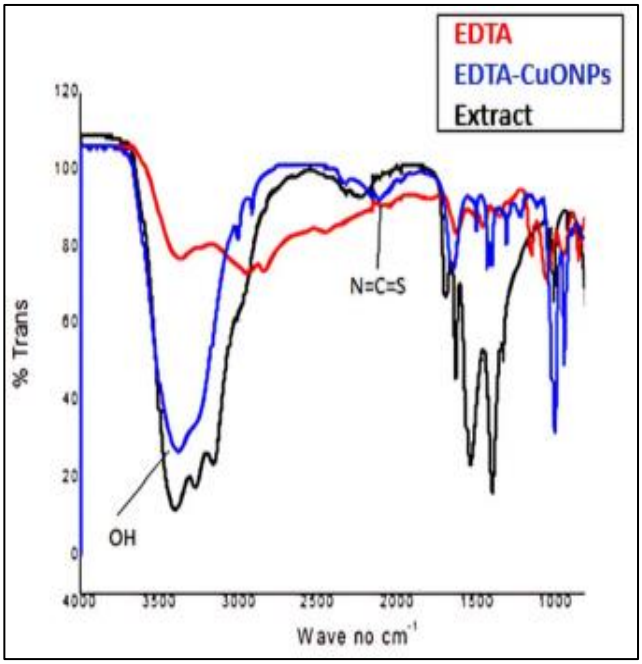

Fig. 8. FTIR spectra of EDTA-CuONPs

3.6 Zeta potential

Size distribution and stability of EDTA-CuONPs were further studied by Dynamic Light Scattering (DLS) and Zeta Potential, the DLS histogram shows that the average size of nanoparticles is less than 80 nm, and particles are highly dispersed, the diameter of particles is observed in 0 to 150 nm range, Polydispersity index was calculated to be 0.25 or less which indicates that the nanoparticles are highly stable and easily dispersed in solution. These findings can be lined up with other studies, where zeta potential values are associated with highly stable nanoparticles, and such particles act as better sensors (Liu et al., 2020). Hence, stable EDTA-CuONPs highlight their application in sensor devices, where size and dispersion conditions are critical for sensor functionality.

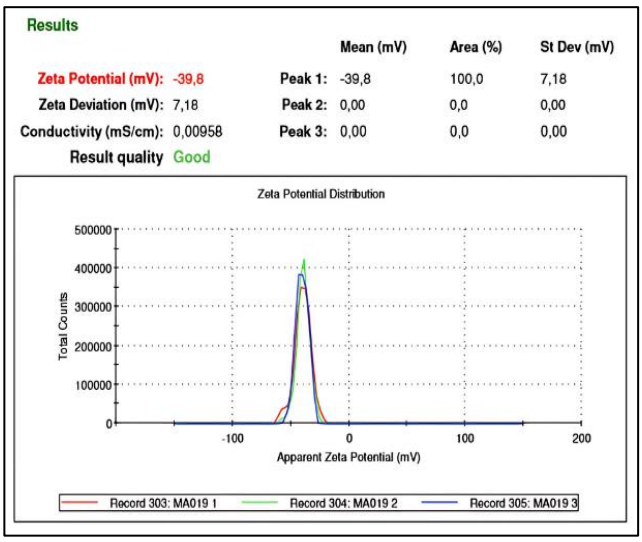

Fig. 9. DLS Zeta Potential Graph of EDTA-CuONPs In Red Color. Blue Color Is Used For The Sample Of CuONPs Without EDTA And Red Color Indicates The Plant Extract.

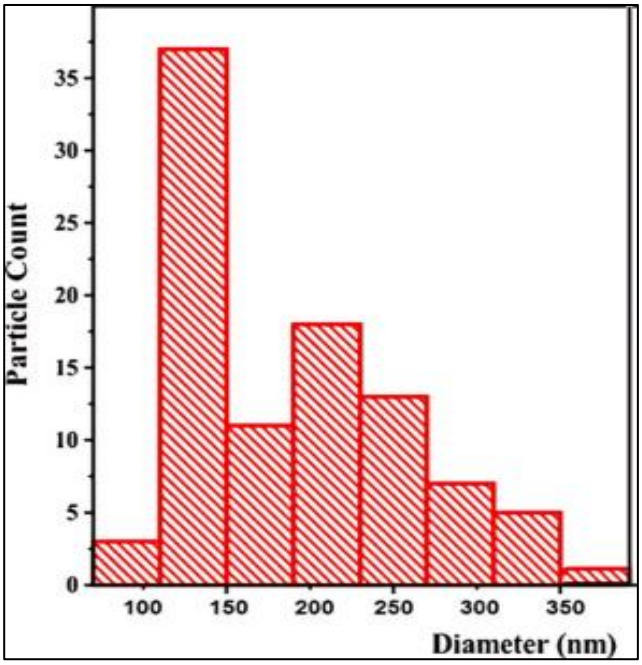

Fig. 10. Size Distribution Graph Of EDTA-CuONPs From Dynamic Light Scattering DLS data

3.7 Applications In Real Samples

We have tested the EDTA-CuONPs over several batches of IFs and Phytoestrogens. The analytical properties of EDTA-CuONPs for the selective removal of isoflavones and phytoestrogens from biological samples such as wastewater, environmental water, serum, and urine were studied under optimized conditions. Our results and values show consistency in the synthesized sensor over repeated trials. It should be noted that consistency depends on the synthesis method of CuONPs, the concentration of phenolic compounds, the size and shape of nanoparticles, pH, and environmental conditions. However, EDTA-CuONPs have a stable size with neutral pH with an effective synthesis method, which makes them highly eligible to remove IFs and Phytoestrogens selectively with consistency.

3.8 Quantifying The Sensor's Detection Limit For Phenolic Compounds

The detection of the EDTA-based CuONPs sensor refers to the lowest concentration of phenol that can be detected with the highest accuracy and precision. Our results show that this detection limit is accurate for the sensor's application in environmental and real samples. Concentration range of 10–100 µM, linear. LOD and LOQ were determined by the ratio between the standard deviation of 10 blank readings and the slope of the 3rd and 10th calibration curves, respectively. The LOD and LOQ values of isoflavones in actual water samples were 0.36 and 1.68 µM, respectively. While the LOD and LOQ for the detection of phytoestrogens in biological samples were 0.45 and 1.21 µM, respectively, the LODs for the

detection of isoflavones in aqueous environments and phytoestrogens in serum and urine were acceptable.

3.9 Percentage Recovery of Isoflavone and Phytoestrogen in Water Samples

Table 1 represents the %Recovery from added and spiked samples.

Table 1

Determination of Percentage recovery of Isoflavone in real samples, Condition (2mL EDTA-assisted CuONPs, real samples diluted 3 times, number of replications=3 and reaction time 5 minutes)

Samples	Isoflavone added in (µM)	Isoflavone recovered in (µM)	Recovery (%)
Groundwater	30.29	31.19 ± 0.9	96.5
Lake water	63.69	65.7 ± 0.13	101.4
River water	220.29	222 ± 0.14	99.3
Tap water	160.69	162.9 ± 0.9	98.7
Blood Serum	63	66±0.11	98.7
Urine	108	111±0.19	101

Table 2

Characteristic Features and Applications of EDTA-CuONPs

Characteristic Feature	EDTA-CuONPs
Capping Agent	EDTA
Accelerating Agent	NaOH
Visibility	Naked Eye (Color change)
Reaction Time	Within minutes
Stability	One week
UV-Visible Spectrum	320 nm
Crystalline Nature	FCC
Particle Shape	Clear and grain-like
Average Particle Size	20 Nm
LOD	0.36 µM
LOQ	1.68 µM
Linear Range	µM to µM
Interference/Detection	Phenolic Compound Isoflavone and Drug Phytoestrogens
Real Samples	River, Tap, Lake, groundwater, and Blood serum Urine
Environmental Application	Isoflavone Detection from Contaminated Samples
Biological Application	Phytoestrogens Detection from patients' samples and wastewater

4. Conclusion

In this research, a facial and cost-effective scientific approach was carried out for synthesizing EDTA-CuONPs through the cost-effective, green, and time-saving method, which has not been reported previously. The synthesized EDTA-CuONPs exhibit promising properties and characteristics, including uniform dispersion, high crystallinity, controlled size, and stability under neutral pH conditions. These EDTA-CuONPs have demonstrated excellent application as sensors due to their high surface area, stability, and strong interaction with phenolic compounds. EDTA-CuONPs showcase their ability to detect IFs and Phytoestrogens with high precision and accuracy. Future studies could optimize the synthesizing method to further analyze different metals and compounds from real water samples. In the future, we can expand their application as sensors to work as real-time detection devices for environmental samples and healthcare monitoring.

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