



Production of copper nanoparticles using alpha-amylase enzyme by an electrochemical deposition method



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HIGHLIGHTS

- Copper nanoparticles were produced using an electrochemical method with alpha-amylase from camel saliva.
- The size and shape of CuNPs were improved after adding alpha-amylase, resulting in stable 44nm particles.
- The used process is eco-friendly, rapid, and non-toxic, utilizing CuSO₄ and varying enzyme concentrations.
- Enhanced CuNPs dispersion and stability were achieved through coordination bonds with the enzyme.

ABSTRACT

This study aims to explain the effect of adding the bioactive substance (alpha enzyme) to the electrolyte solution using electrochemical deposition. Through which copper nanoparticles are manufactured, the originality of the research lies in the presence of this addition as a new method in manufacturing nanoparticles in an electrochemical manner, as the alpha-amylase enzyme is extracted from camel saliva, and the product has been diagnosed using (EDX) and scanning electron microscope (FE-SEM) techniques. , Dynamic Light Scattering (DLS) technology, and Zeta potential. The proposed method is an easy, inexpensive, and environmentally friendly method where the alpha enzyme was used as a feedstock at concentrations of (0.25, 0.50, 0.57, and 1) g added to a solution containing the ideal sample obtained through the electrochemical precipitation process before adding the enzyme, as the solution before The addition process contains concentrated sulfuric acid (H₂SO₄) at concentrations of (20, 30, 40, 50 and 60) g/ml. The regular shape and stable distribution can be observed after the addition compared to the shape of the particles before the addition in terms of shape and size. The process was carried out under constant conditions, including the current. Direct, voltage and temperature, and after the addition process, sulfuric acid was dispensed with, while the process conditions with the addition were at a temperature of 37 degrees Celsius and an incubation period that lasted a full hour before the electrodeposition process was carried out in accordance with the enzyme activity conditions, where a nanosized copper powder of a granular size was obtained. Approximately 44 nanometers. It is worth noting that the nanoparticle size was modulated using the experimental conditions, e.g., pH, reducing step, enzyme amount, or incubation time. This controlled synthesis allows the preparation of CuNP bionanoparticles at Cu 3.13 powder at a concentration of 80 g CuSO₄ + 0.50 α-amylase.

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1. Introduction

Nanobiotechnological approaches have received intensive attention in therapeutics and diagnosis because of their unique physicochemical properties that revolutionize medical treatment with more potent and less toxic nanoparticles [1]. In this context, copper (Cu) and its alloys are of significant importance for many branches of modern engineering due to their versatile properties, such as high electrical and thermal conductivity and high resistance to corrosion, in addition to their usage as disinfectants due to their antibacterial as well as antiviral properties [2], which have been enhanced with the production of this material in the nanoscale. Nonetheless, the increase in concerns related to the environmental impact has led to the development of eco-friendly processes, where the nanoparticles were obtained using biological approaches. In particular, copper belongs to the group of light transition metals, and thus, Cu NPs cannot just be obtained directly from simple copper salts. It needs

capping agents, such as surfactants, to control the size of particles, which effectively reduces copper salts and provides outstanding stability against agglomeration [3].

Electrodeposition is more effective than physical and chemical methods for obtaining nanomaterial, and that can be credited as a challenge due to the complexity of the biological extracts that pose a barrier to the elucidation of the reactions and mechanism of formation that occur during the synthesis [4]. Therefore, the synthesis of copper nanoparticles is essential due to their usefulness in many applications [5]. The properties of such materials can be engineered by controlling the dimensions via physical, chemical, or biological methods, which only produce small amounts of nanomaterials [6].

Inorganic nanomaterials are conventionally synthesized under harsh environments, such as extreme temperature, pressure, and pH. These methods are eco-unfriendly, expensive, toxic, and cumbersome, and they yield bigger particles that agglomerate due to not being capped by capping agents. In contrast, biological synthesis of inorganic nanomaterials occurs under ambient conditions, including room temperature, atmospheric pressure, and physiological pH, and it is reliable, eco-friendly, and cheap [7]. Recent advancement in this field includes the enzymatic method of synthesis, suggesting the enzymes/proteins to be responsible for the nanoparticles' formation.

Recently, synthesized and produced copper nanoparticles using the purified alpha-amylase enzyme secreted by the salivary glands at neutral pH of single-humped camels located in Basra, southern Iraq, where a live amount of camel saliva was taken in the Safwan district of Basra. In cooperation with specialized veterinarians and under the supervision of the Veterinary Medicine Hospital, the procedures began by giving camels a salty or sweet substance to increase the amount of saliva in their mouths. In this regard, their salivary secretions play an important role in the regulatory ability, pH balance, and alkalinity, and they have a high salivary content because they are ruminants that eat grasses and plants. In particular, enzymes are found only in saliva, and in order to be able to use them for digestion, the camel's body expels some of the contents of the stomach into the mouth, where it is chewed and broken down more and more. With this process, stomach contents mix well with saliva, and sometimes, the pH value of saliva changes and becomes more acidic or alkaline depending on environmental factors, such as diet, stress levels, and the circadian rhythm of saliva. The α -Amylase (EC 3.2.1.1) catalyzes the hydrolysis of starch into simple sugars, where it was found that the enzyme activity in camel serum was higher (2325 units/L) than that in camels in cattle serum with only 77 units/L p3. The development of protocols for the synthesis of copper nanoparticles has been an important area of outstanding research due to its enormous applications in diverse fields, including catalysis, optics, and medical diagnostics and therapy. In this paper, the alpha-synthesis method for the production of copper nanoparticles, in which electricity is used as a controlling force. Particularly, electrochemical synthesis occurs by passing an electric current between two electrodes separated by an electrolyte. Therefore, the synthesis takes place at the electrode/electrolyte interface [8,9].

This work presents a simple, novel, cost-effective, and environmentally friendly approach by creating a new class of biotechnological materials. Scientific developments in the current decade are of great importance in preparing nanoparticles and controlling particle size, shape, and morphology [10]. In the proposed method, improvements have been made to the electrochemical deposition process using a biological material through which nanoparticles can be synthesized and produced. In addition, the proposed method aims at making the electrochemical deposition process environmentally friendly by eliminating acids that are harmful to health, such as sulfuric acid, where the utilization of the alpha-amylase enzyme does not produce large quantities of unwanted secondary materials and it is a non-toxic material. Furthermore, the suggested method controls the growth of crystals, their stability, and the method of dispersion by electrostatic dispersion across the surface of amino acids, thereby improving the properties [11]. The aim of research that to improve the electrochemical deposition process by using a biological material through which nanoparticles can be synthesized and produced. Making the electrochemical deposition process environmentally friendly by eliminating acids that are harmful to health, such as sulfuric acid, and using the enzyme alpha-amylase does not produce large quantities of unwanted secondary materials and is non-toxic.

Controlling the growth of crystals, their stability, and the method of dispersion by electrostatic dispersion across the surface of amino acids, thus improving the properties. So, in this research, we study the effect of adding alpha-amylase enzyme on the production of copper nanoparticles by electrodeposition from copper sulfate solution compared with the traditional method of production from sulfuric acid solution. During the last decade, metal nanoparticles (MnNPs) have gained immense popularity due to their characteristic physicochemical properties, as well as containing antimicrobial, anti-cancer, catalyzing, optical, electronic, and magnetic properties. Primarily, these MnNPs have been synthesized through different physical and chemical methods. However, these conventional methods have various drawbacks, such as high energy consumption, high cost, and the involvement of toxic chemical substances, as compared with the electrodeposition process. First, we will investigate the optimum current density for the production of copper from acidic media by applying different current densities (0.055, 0.104, 0.164, 0.219 and 0.275) mA/cm² in order to get the optimum current density. The copper sulfate concentration was constant in all experiments (100 g/l), and the sulfuric acid 97% concentration was (91.5 g/l) or (50 ml: 1L). In the second part, we prepare the same solution of copper sulfate. Still, instead of sulfuric acid, we use (1,2,3 and 4 g/l) of alpha-amylase enzyme and investigate the optimum condition by testing the FE-SEM, Energy Dispersive X-Ray analysis (EDX).

2. Experimental details

2.1 Powder preparation by electrodeposition

In all experiments, the solution prepared from Copper sulfate pentahydrate (CuSO₄·5H₂O) 99.94%, distilled water, sulphuric acid H₂SO₄ 97%, while Sodium tartrate (CuH₄Na₂O₆·2H₂O), and Sodium bicarbonate (NaHCO₃) was used for washing the copper powder. A commercial copper plate was prepared and was cut to dimensions (80 × 30 × 3) mm³ for use as

an anode, and a plate of stainless steel 316 was used as a cathode in dimensions ($100 \times 25 \times 2$) mm³ as shown in Figure 1 (a and b). The cut process is done by a mechanical cutter, and the chemical composition of the stainless steel cathode and copper anode is shown in Table 1. Power supply type Parmer, English-made maximum value of the current (10 A) and the voltage (50 Volts). Stirring using Teflon coated magnetic stirrer bar, Baker glass- heat resistant, Sensitive balance (sartorius German- made accurately 10.0001), pH meter to measure pH of solution type (Philips/pw 9421 ph meter), Electric drying oven at 80°C for one hour.

Table 1: Chemical composition of stainless steel 316 and copper

Element	Fe	V	Cr	Ni	Mo	Mn	Mg	Al	Si
Cathode con.%	64.79	0.0787	17.16	11.79	1.423	1.613	0.419	0.595	0.7
Element	Cu	Al	Si	Mg	P	S	Ti	V	
Anode Con.%	97.200	0.945	0.297	0.310	0.022	0.0134	0.0299	0.0196	

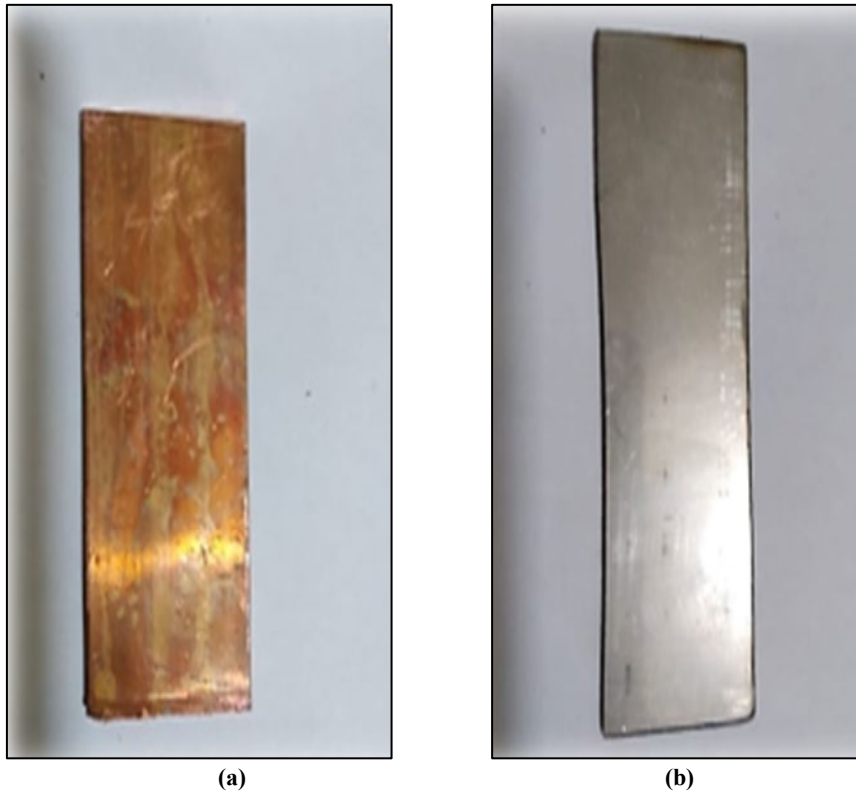


Figure 1: (a) Electrode of copper at the anode, (b) Electrode of stainless steel at the cathode

2.2 The electrodeposition process without additives amylose enzyme

The electrolytic solution preparation produces copper powder, which is a mixture of copper sulfate with distilled water and sulfuric acid (all chemicals were of analytical grade and were supplied by Merck), acting as the source of copper ions and increasing the conductivity of the electrolytic bath. More specifically, the procedures were done first by adding 25 ml of sulfuric acid 97% to 100 ml of distilled water gradually with continuous mixing until the sulfuric acid was well mixed in the distilled water. The copper sulfate was added with the required quantities according to each experiment. Moreover, they are well mixed with a magnetic mixer to obtain complete solubility and clear solution continuously operating period. The electrodeposition process was achieved by immersing the copper anode and stainless steel cathode to the level that obtains the required current density. Then, both the anode and cathode were connected to the power supply, as shown in Figure 2, in order to complete the electrodeposition circuit.

2.2.1 Effect of current density

The effect of current density on the electrodeposition process of copper powder was studied by applying the current (1,2,3,4 and 5) Amp. In each experiment, the rest of the variables were fixed, as illustrated in Table 2, and then the best current density was determined to be adopted as an optimum operating condition.

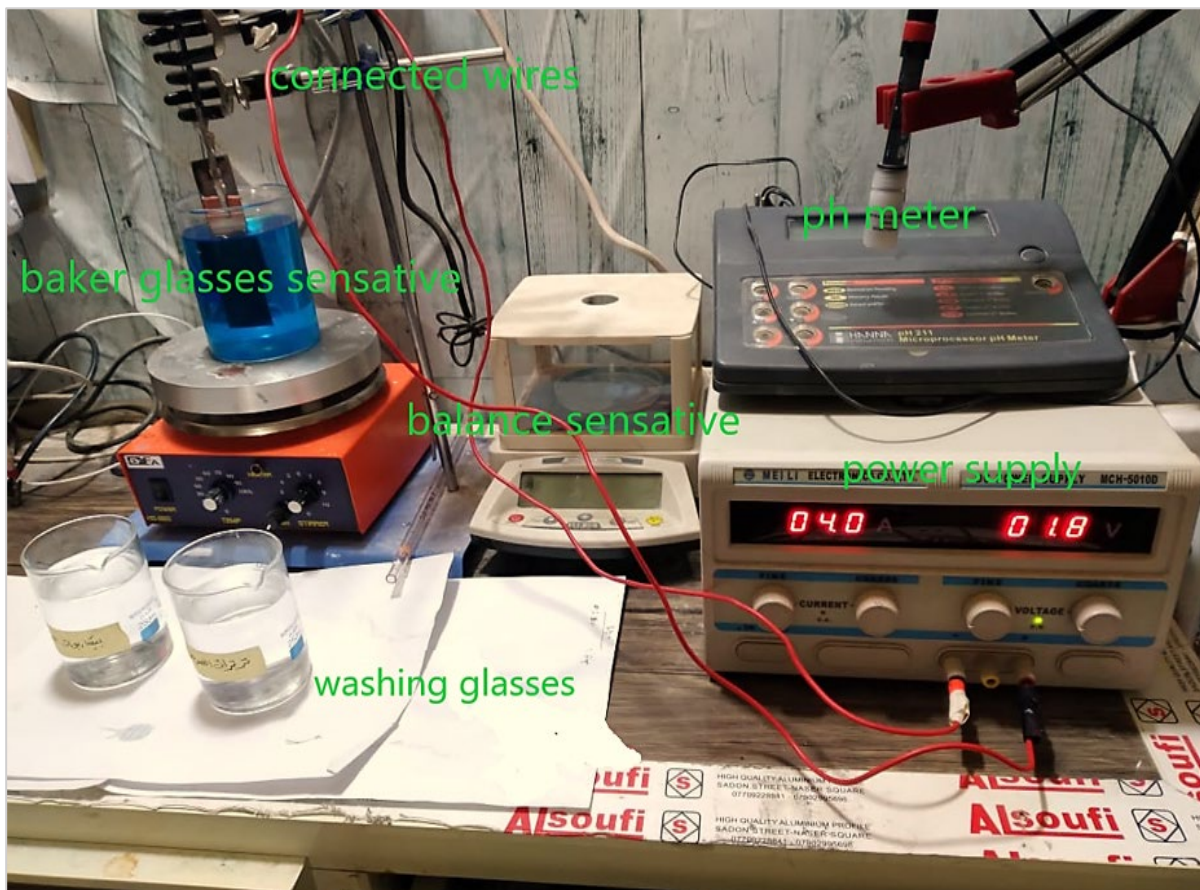


Figure 2: Electrodeposition system

Table 2: The operating conditions for the effect of current density

Current(Amp)	1.0	2.0	3.0	4.0	5.0
Potential(V)	1.5-2.2	1.9-2.8	2.3-3.9	3.5-5.1	4.8-7.5
CuSO ₄ con.(g/l)	100	100	100	100	100
H ₂ SO ₄ con.(ml/L)	50	50	50	50	50
pH change at experiment period			0.31-1.46		
Time(min)			60 min		
current density at cathode (mA/cm ²)	0.055	0.109	0.164	0.219	0.275
Copper Powder Weight(g)	0.99	2.16	3.13	4.03	5.00

2.3 Alpha-amylase enzyme

2.3.1 Chemical structure of the alpha amylase enzyme

Enzymes are biocatalysts that speed up the reactions taking place inside the cell. They are widely used in industries, scientific research, and clinical diagnostics. Specifically, they increase the rate of reaction by lowering the activation energy required to convert [12], where the enzyme plays the role of counter ions, knowing that the ideal pH for the salivary amylase enzyme is (6.6-6.8) pH at a temperature of 37 °C substrates into the products. The catalysis by an enzyme is influenced by the nature of the medium, the substrate, the enzyme concentration, the temperature, the pH, and the presence of activators and inhibitors. In this regard, nanoparticles are solid dispersion particulates [13]. The optimum activity of α -amylase was found to be in the pH range of 4.5 to 7.0. Decreasing the pH of enzyme solution below this range results in a decrease in the enzyme activity. The α -Amylase is one of the most common enzymes used in vitro nanoparticle synthesis. It acts as both a reducing agent and a capping agent, and because enzymes are protein compounds with large molecular weights, when they are hydrolyzed, they give amino acids. Therefore, amino acids are the building blocks of proteins and enzymes [14]. Similar to other ruminants, the secretion of saliva in camels is large because it contains an amount of amylase enzyme, whose secretion rate in the event of water availability reaches 21 liters per day, and its rate decreases until it reaches 0.64 liters in thirsty camels α amylase, as illustrated in Figure 3 [15].

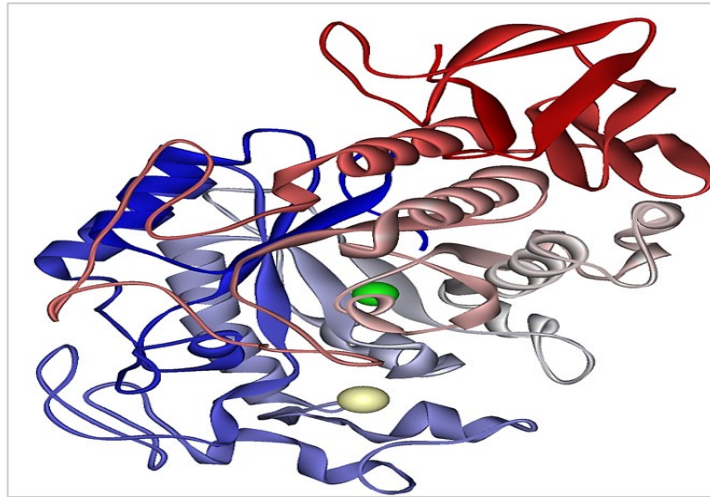


Figure 3: Structure of the alpha-amylase enzyme [15]

2.3.2 Extraction method of the alpha-amylase enzyme from saliva

2.3.2.1 Ammonium sulfate precipitation

Ammonium sulfate was added to the crude enzyme (supernatant) with different saturation ratios of (70%). The mixture was mixed gently on a magnetic stirrer for 20 minutes at 4 °C. Then, the precipitated proteins were dissolved in a suitable volume of 0.5 M Tris-HCL buffer at pH8 (2.1, 5.1 °C). The enzyme activity and the protein concentration were estimated. Under cooling conditions (4 °C), the enzyme solution was dialyzed after precipitation with ammonium sulfate using distilled water (DW) for 24 hours, and the DW was changed four times. Then, the enzyme activity, the specific activity, and the protein concentration were measured [16].

2.3.2.2 Purification by ion exchange chromatography (2x 25cm) column

The DEAE-cellulose column was prepared according to Whitaker and Bernard (1972) by suspending 20 g of resin in 1L of D.W. Then, the mixture was left to settle down and washed several times with D.W. until it had a clear appearance. The suspension was filtered throughout Whatman No. 1 using a buchner funnel under discharging. The resin was re-suspended in 0.25M sodium chloride and sodium hydroxide solution. The suspension was filtered again, as mentioned above, and washed several times with 0.25 M hydrochloric acid solution and followed by distilled water before it was equilibrated with 0.05M phosphate buffer pH 7. Next, the enzyme solution obtained from the previous step was applied to the DEAE-cellulose column (2x25cm) equilibrated previously with 0.05M phosphate buffer pH7. Then, the column was washed with an equal volume of the same buffer while the attached proteins were stepwise eluted with gradual concentrations of sodium chloride (0.1–1M). The flow rate throughout the column was 30ml/hrs., and the absorbance of each fraction was measured at 280nm using a UV-VIS spectrophotometer. After that, the enzyme activity was determined in each fraction.

The Sephadex G-150 was prepared as recommended by Pharmacia Fine Chemicals Company. In particular, a quantity of the Sephadex G-150 was suspended in 0.05 M phosphate buffer pH7, subjected to heating at 90 °C for 5 hours to ensure the swelling of the beads, degassed, and packed in a glass column of (2×40 cm). Then, it was equilibrated with the same buffer. Next, a concentrated sample obtained from the ammonium sulfate step was applied to the column. Elution was achieved at a flow rate of 30mL/hr, and the same buffer was used for equilibration. The absorbance of each fraction was measured at 280 nm. The enzyme activity was also determined in each fraction, and the protein concentration was determined using Bradford (1979).

2.3.2.3 The potassium phosphate buffer (0.05 m, ph 7.0)

This buffer was prepared by dissolving 0.87g of K_2HPO_4 in 100 ml D.W. and 0.68g of KH_2PO_4 in 100 ml D.W. separately. Then, 61 ml of K_2HPO_4 was mixed with 39 ml of KH_2PO_4 solution. The pH was adjusted to 7.0, and the volume was completed to 200ml using D.W.

2.3.3 Effect of concentration of alpha amylase enzyme

At this stage, we studied the effect of adding amylase with the concentrations of (0.25, 0.50, 0.75, and 1) g with incubation for one hour to find out the effect of this enzyme on the electrochemical deposition process. In this regard, it is worth mentioning that this is the first time an animal material is used, and it is introduced into the metallurgy technique using biochemistry science under pre-specified standard conditions, where we extracted the amylase enzyme from camel saliva. After obtaining the amylase liquid, we examined the pH, and it was about 6.4. Then, to maintain the effectiveness of the enzyme for long periods, it was converted into powder by means of a device, namely, the (christ freeze dryer lypholizel chermany company) under -72 °C and 0-0010 mbar conditions, where the drying period took about five days. The process took place in three stages. First, the best concentration of copper sulfate was mixed. It was obtained from the electrochemical precipitation process. It was dissolved in 250 ml of distilled water, after which the amylase enzyme was added in concentrations and the solution was kept for an hour at a temperature of 37 °C in a water bath with mixing to homogenize the solution, and the results were obtained as shown in Table 3.

Table 3: The effect of adding amylase concentrations (with incubation)

CuSO ₄ con.(g/l)	80			
Amylase concentration(g)	0.25	0.5	0.75	1.00
Potential(v)	22-15			
pH	3.7-4			
Time(min)	60			
Current(amp)	3			
Electrical conductivity(ms)	4.15	11.5	4.6	3.3
Produced Copper Powder Weight (g)	3.02	3.04	2.99	2.11

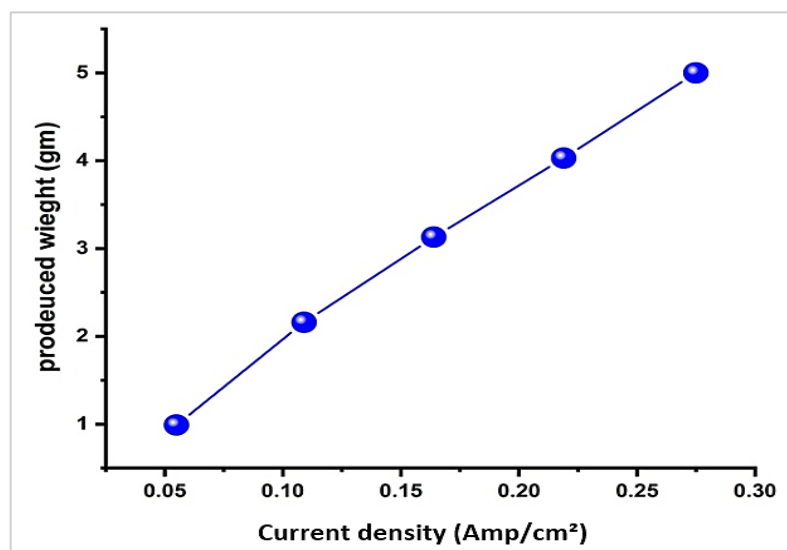
3. Washing and drying powder

In the beginning, washing powder, whether it was before or after the addition, was collected using distilled water. Then it was treated with a solution of sodium bicarbonate (0.05%) and sodium tartrate (6 g/L to protect the powder against subsequent oxidation after completion of the process of washing. After that, the powder was washed with absolute alcohol 99%, and the reason for this step, as we noticed through the experiments, is that the presence of washing materials or water may later cause oxidation of the copper powder. The powder was filtered and dried inside the oven at a temperature of (80 °C), and then it was weighed [17].

4. Results and discussion

4.1 The effect of current density

To demonstrate the effect of current densities on the process of electrical deposition of copper powder, practical experiments were conducted based on different values of current densities, including (0.055, 0.109, 0.164, 0.219, and 0.275) amp/cm², consisting of copper sulphate and sulfuric acid at a constant temperature with a distance of 3 cm between Electrodes. The copper powder was obtained on the cathode electrode, where we noticed an increase in the weight of the deposited copper powder by increasing the value of the current density, as shown in Figure 4, which shows the effect of the current density on the weight of the deposited powder. In particular, the current efficiency reaches about (98%) at (3 amp/cm²), while it reaches (65.7%) at (2 amps/cm²), then it decreases to (60.9%) at (4 amp/cm²). Increasing the values of current densities makes the solution more saturated with ions due to the increased rate of current passing through the electrolyte cell, which leads to the deposition of copper powder on the surface of the cathode electrode. The powder then begins to precipitate at high current densities. The higher the current density, the smaller the particle size of the deposited powder.

**Figure 4:** The effect of the current density on the weight of the deposited powder

The increase in the values of current densities makes the solution more saturated with ions because of the increased rate of the current passing into the electrolyte cell, which leads to the deposition of copper powder on the surface of the cathode electrode. Then, the powder begins to precipitate at high current densities. The greater the density of the current, the smaller the size of the granules of the precipitated powder and the greater its looseness. When the current density increases, the number of separated crystal nuclei on the cathode increases, and this leads to the separation and formation of fine powder particles [18].

The morphology of the powder through FE-SEM examination showed significant dispersion with increasing current density. In this regard, morphological analysis shows that at all current densities except $j=0.209$ A/cm² as shown in Figure 5 (a, b, c, d and e). Typical particle size distributions of copper powders obtained at five different current densities using a dynamic light scattering (DLS) test show the correlation curve, which should be smooth to study the particle distribution. Opposite of fluctuations in the intensity of light scattered by particles. The measured particle size varied as in Table 4 and was better with increasing current density.

Table 4: The change of particle size measured by (DLS) with current density

Current density (mA/cm ²)	Particle size (nm)
0.055	121.9
0.109	118.3
0.169	108
0.209	106
0.275	104.1

Typically partical size distribution of copper powder obtain at five differnet current density as shown in Figure 6 (a, b, c, d and e), by dynamic light scattering (DLS) test is the correlation curve which must be a smooth to investigation the particle distribution opposite fluctuations in the intensity of the light scattered from the particles.

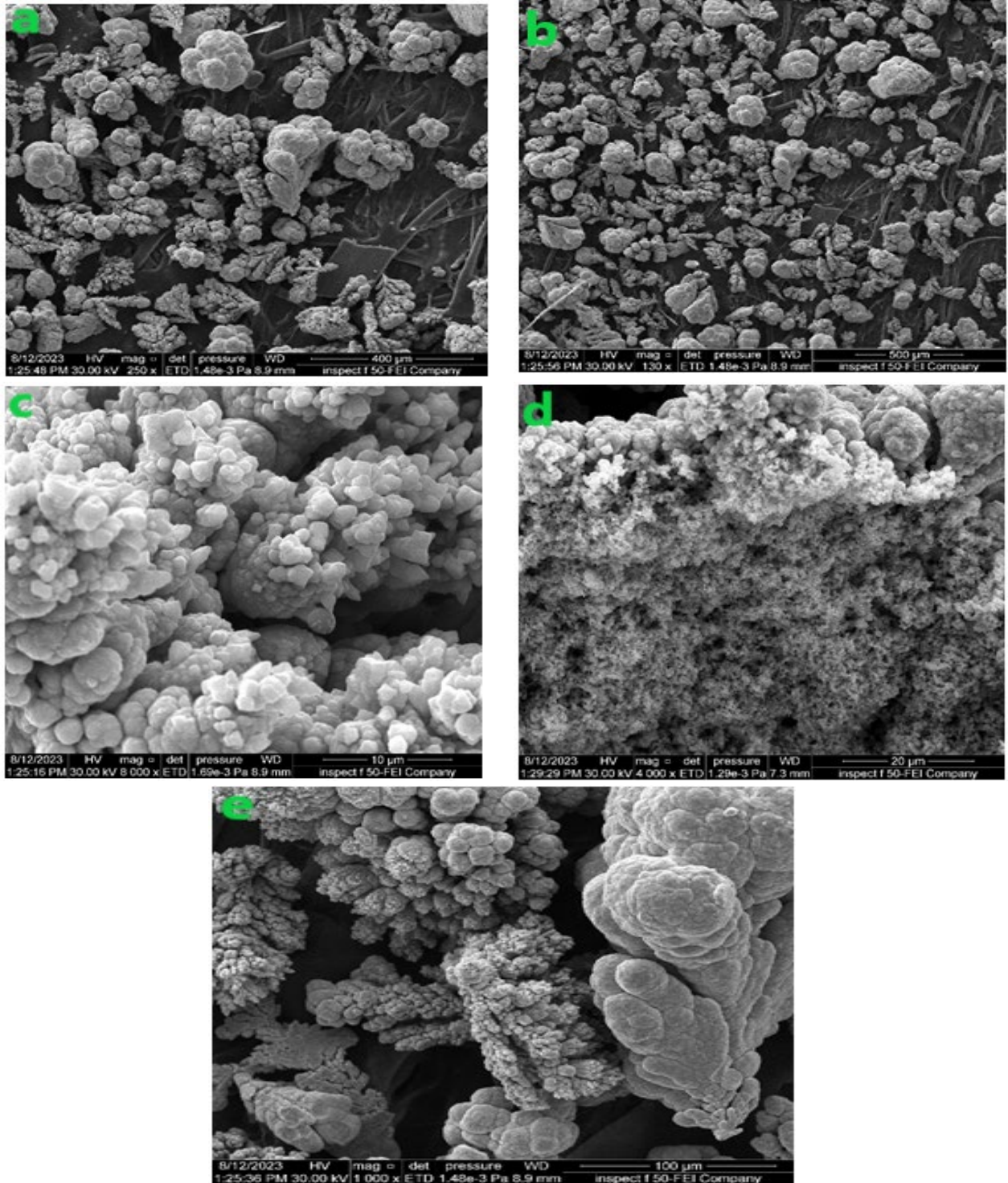


Figure 5: Morphology of copper powder by FE-MES at current density (a) $j=0.055$, (b) $j=0.109$, (c) $j=169$, (d) $j=0.209$ and e) $j=0.275$

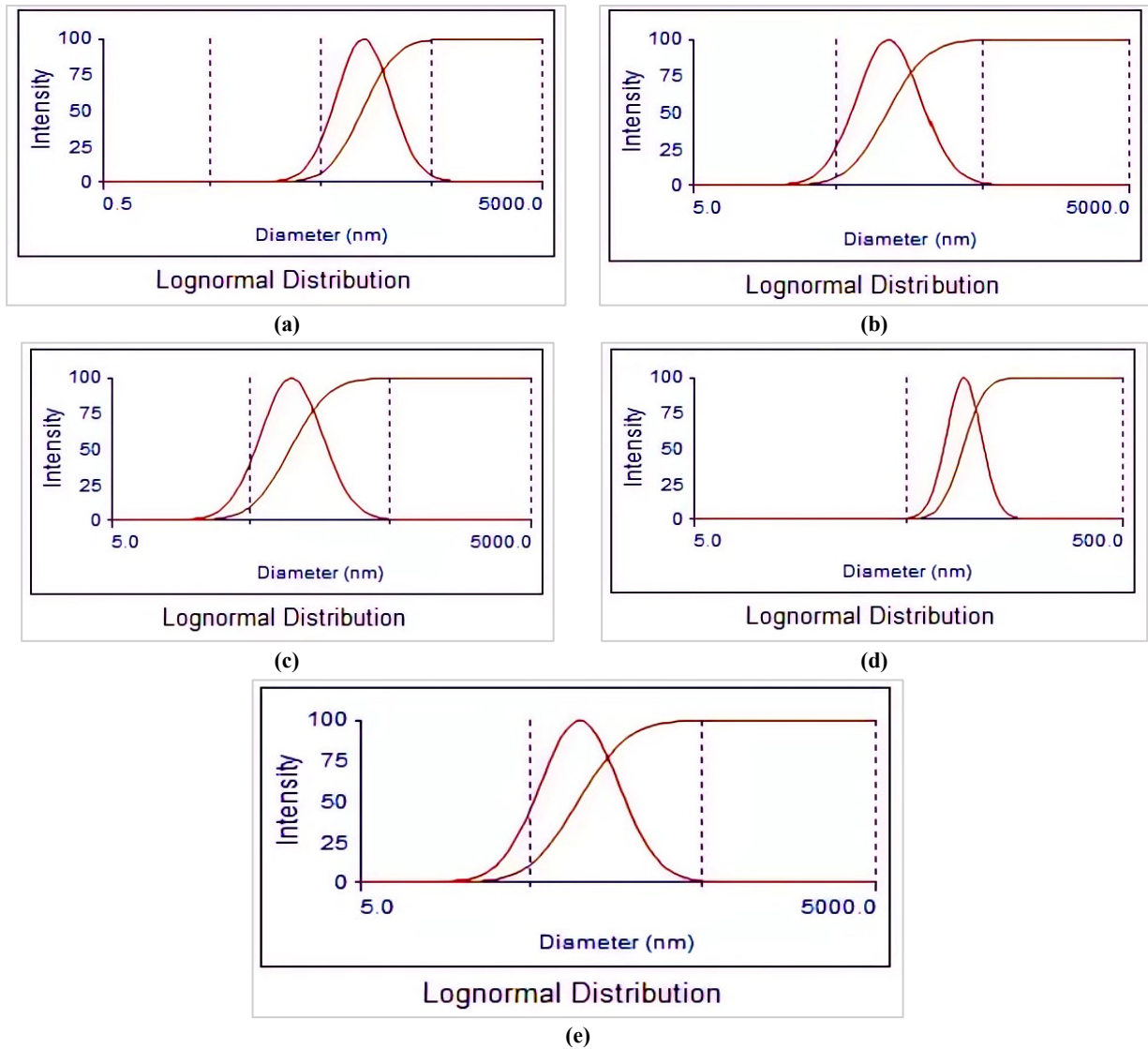


Figure 6: Particle size distribution of copper powder at (a, b, c, d and e) (1,2,3,4, and 5) (Amp/cm²) respectively where deposition time of one hour in 100 g/l CuSO₄+ 50 ML/L H₂SO₄ solution

4.2 The effect of additive alpha amylase

Copper nanoparticles were synthesized using purified alpha-amylase enzymes as an additive to an electrolyte solution consisting of copper sulfate without the addition of sulfuric acid. From Figure 7, we see that the amount of copper produced reached the maximum when the amount of amylase was 0.5 g and then began to decrease until it reached the minimum when the amount of amylase was 1 g. This means that increasing the amount of amylase does not achieve an increase. The weight of copper produced and the ideal sample was at 0.5 g of amylase.

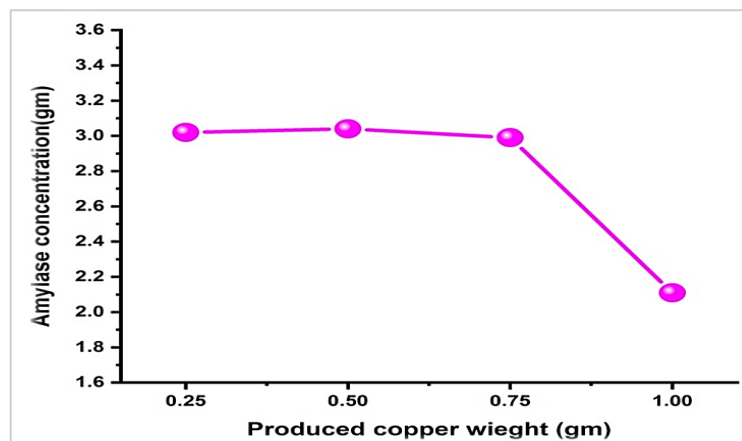


Figure 7: The effect of amylase enzyme quantity addition on the weight of the copper powder produced

Scanning electron microscopy to study the surface morphology of the copper particles shown in Figure 8 (a, b, c and d). We observed that the size range of nanoparticles was about 86.25 and 43.50 nm, respectively. In the first concentrations, the granules were uniform and widely distributed. When the amylase concentration increased to 0.5 g, we noticed that the sizes became softer and took a spherical shape with an increase in the diffusion process. The size of the copper ions became small in the presence of alpha-amylase, and they were stimulated to form stable copper nanoparticles. The crystal structure of Cu NPs powder was successfully synthesized using a biochemical electrophoresis method. It can be said that the addition worked to reduce the porosity, which leads to an increase in the pressure of the particles. It was also found to limit the amount of hydrogen gas released on the surface of the electrode.

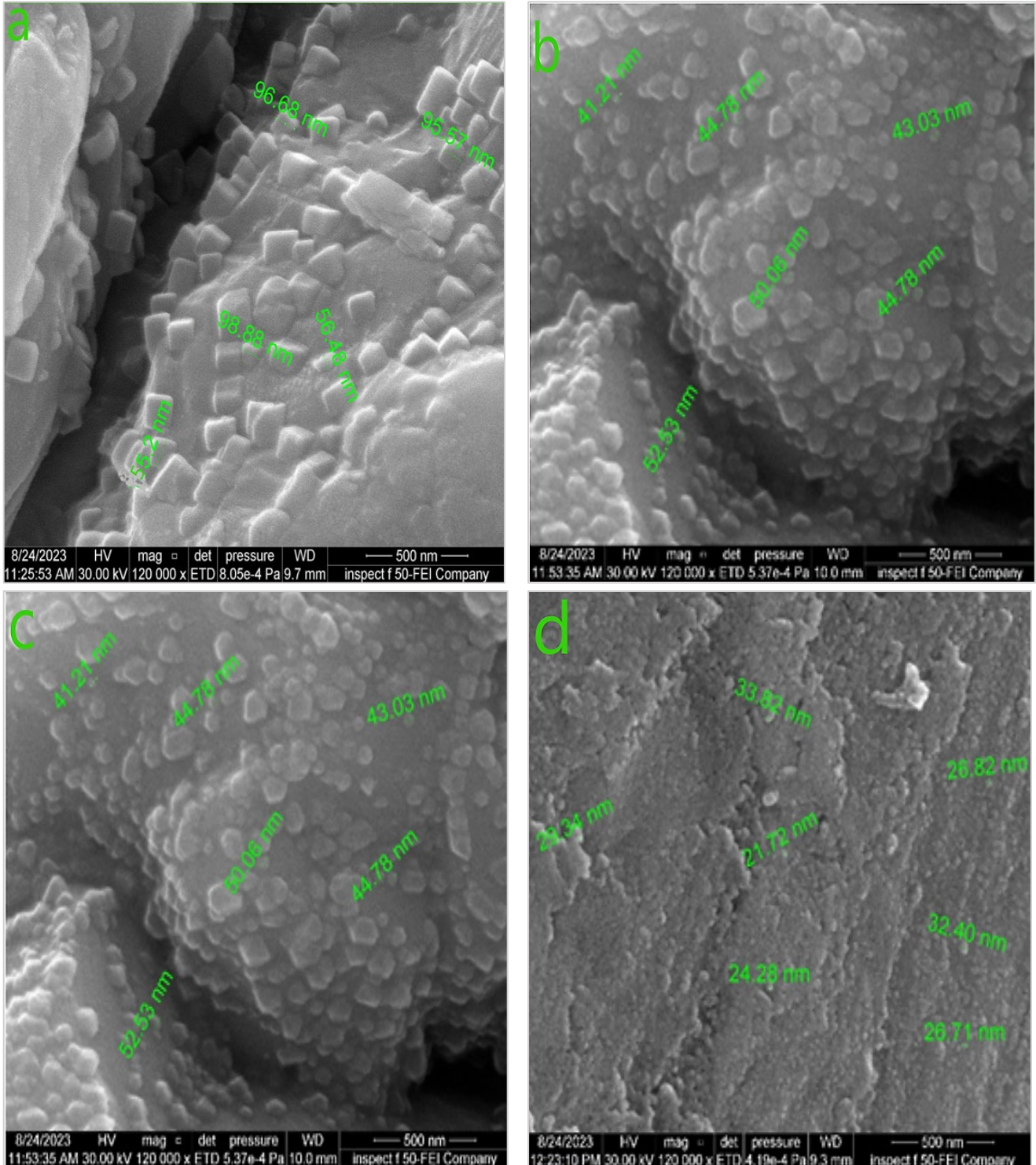


Figure 8: (a, b, c, d) Morphology of copper powder at 100g/L CuSO_4 + amylase additive (0.25a, 0.50b, 0.75c and 1d) g

The enzyme can be considered a molecular compound with a large size and possesses an oscillatory property, which depends mostly on the pH of the electrolyte solution. The mechanism of action of this addition occurs through strong adsorption on the interface between the electrode and the solution during deposition. The number of active sites was reduced, allowing the metal ions to precipitate slowly and be well distributed on the cathode surface, resulting in finer grains [19].

The copper particle size distribution is shown in Figure 9 (a, b, c, d) for amylase incubation under process conditions with added concentrations of (0.25, 0.50, 0.57, and 1) g at 100 CuSO₄ without using sulfuric acid. Smaller particles are formed when the enzyme additive increases until it loses its effectiveness at 76.9, 73.1, 48.1, and 92 nanometers of particle size, respectively, as in Table 5. Adding the enzyme stabilized the metal and kept it inside. The means of chelation are electrical charges, which cause this bound ion to lose its ionic properties and thus become inactive. These compounds encapsulate and bind to the element through more than one excitation. Thus, these vital enzymes work. To reduce agglomeration and wide dispersion of particles, thus reducing particle size.

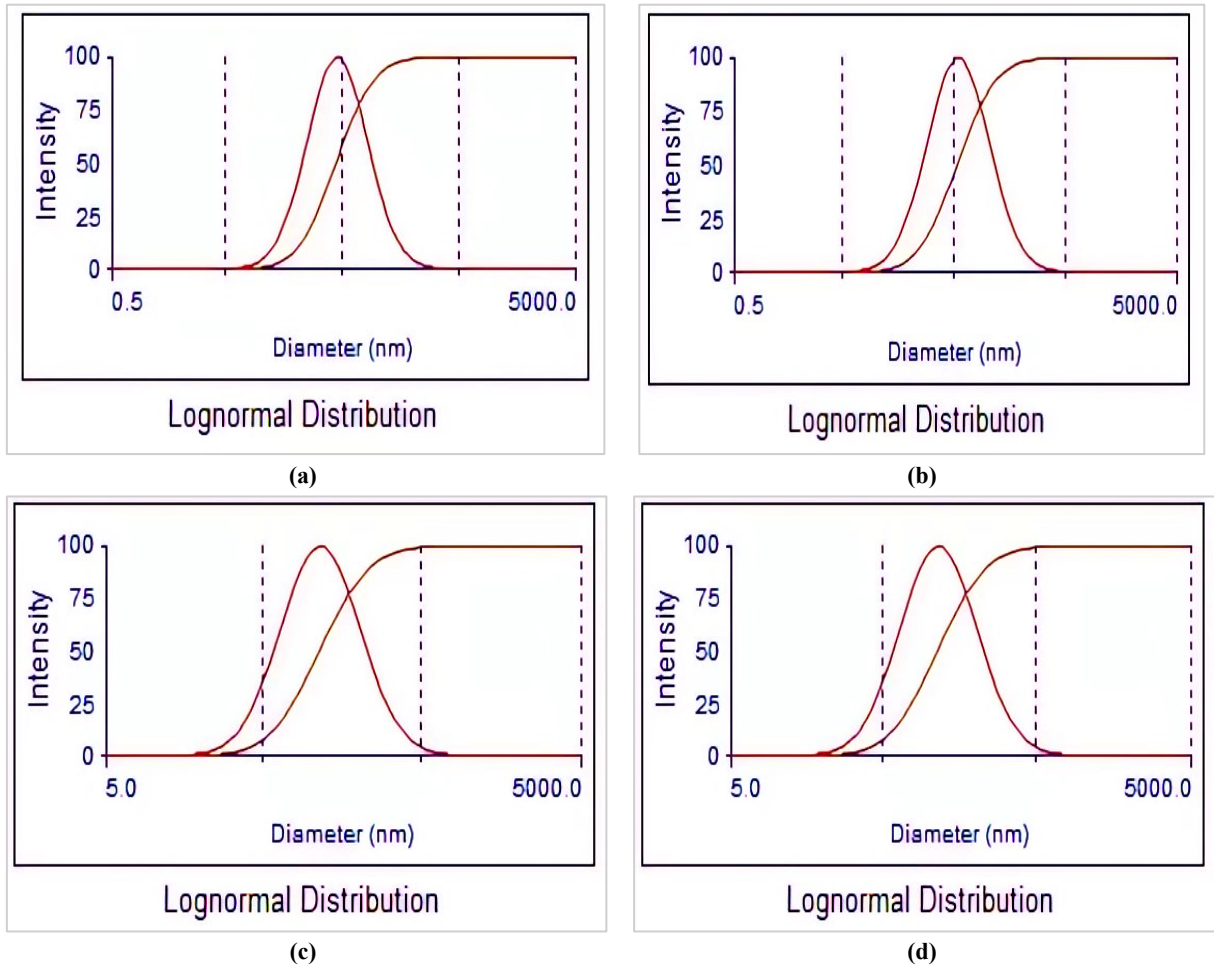


Figure 9: (a, b, c and d) Particle size distributions of copper powders at 0.25, 0.50, 0.75, and 1g of amylase and deposition of one hour, 100 g /L CuSO₂ solution

Table 5: The change of particle size measured by (DLS) with current density

Alph enzyme amylase (g)	Particles Size (nm)
0.25	79.9
0.50	73.1
0.75	48.1
1	92

4.3 EDX test results

The EDX test for copper produced without adding amylase shows strong signals for copper particles that the elemental composition consists of copper with 95.2% as shown Figure 11a and weak signals oxygen concentration of about 4.8% as shown Figure 11b deposit may be due to the fact that most of the resulting Cu₂O dissolve when the concentrated sulfuric acid as shown in Figures (10 and 11) and Table 6 represent the elementals composition without additive.

Table 6: The elemental composition of copper produced without adding amylase

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
O	4.8	0.5	3.8	0.2
Cu	95.2	0.3	96.2	0.5

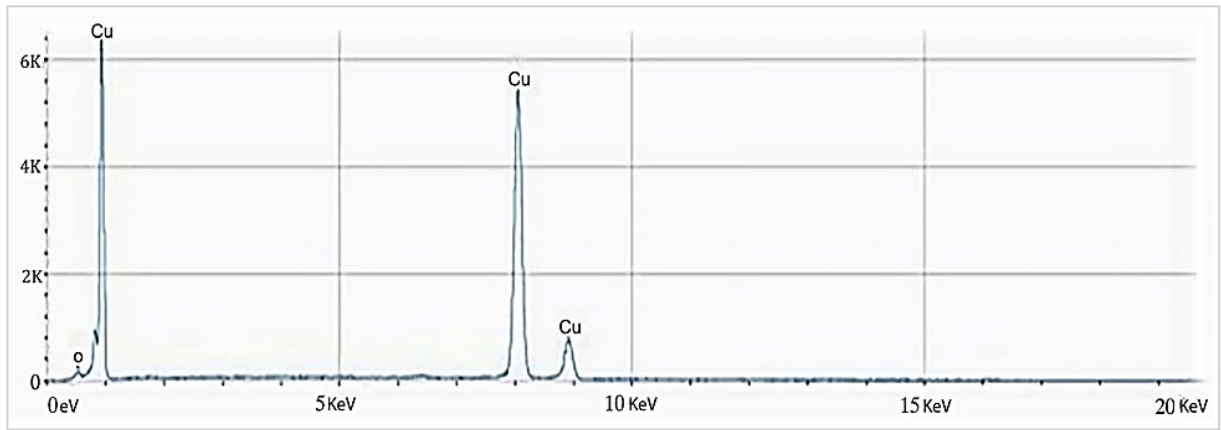


Figure 10: EDX Spectra elements for copper produced without adding amylase at 100 g CuSO₄ without using sulphuric acid

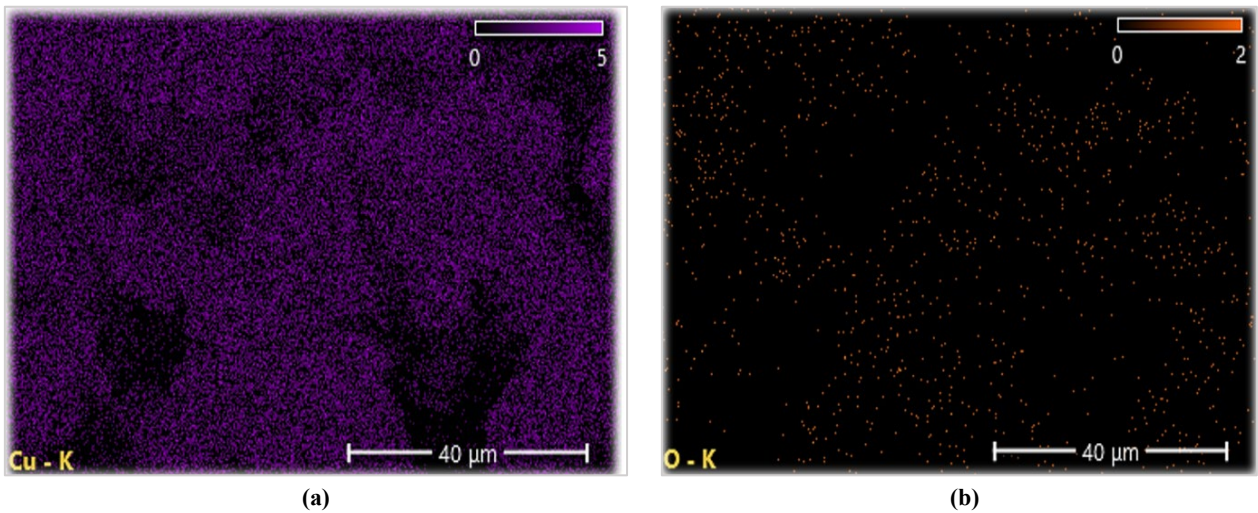


Figure 11: Elemental map for : (a) copper produced without adding amylase at 100 g CuSO₄ and without using sulphuric acid (b) weak signals oxygen concentration

The EDX test for the copper produced with adding amylase shows the appearance of little carbon coming from little amylase stuck with copper, and also, there is little oxide coming from copper oxide as shown in Table 7 and Figure 12 and Figure 13. More importantly, a strong peak of copper particles appears as shown in Figure 13a, larger than when no organic matter is added may be due to the existence of biomolecules that are bound to the surface of CuNPs, as the detection limit for copper is high compared and can obsarfation the weak oxygen as shown in Figure 13b , little carbon as shown in Figure 13c.

Table 7: The composition elements for copper produced with adding amylase

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
C	1.4	0.8	1.2	0.2
O	1.6	0.3	1.0	0.1
Cu	97	0.3	97.8	0.5

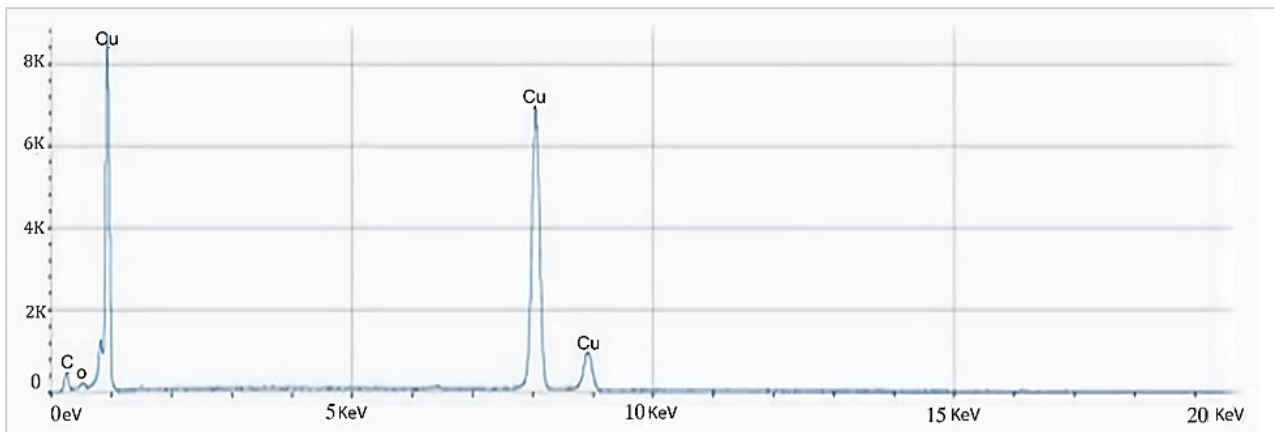


Figure 12: EDX spectra elements for copper produced by adding amylase

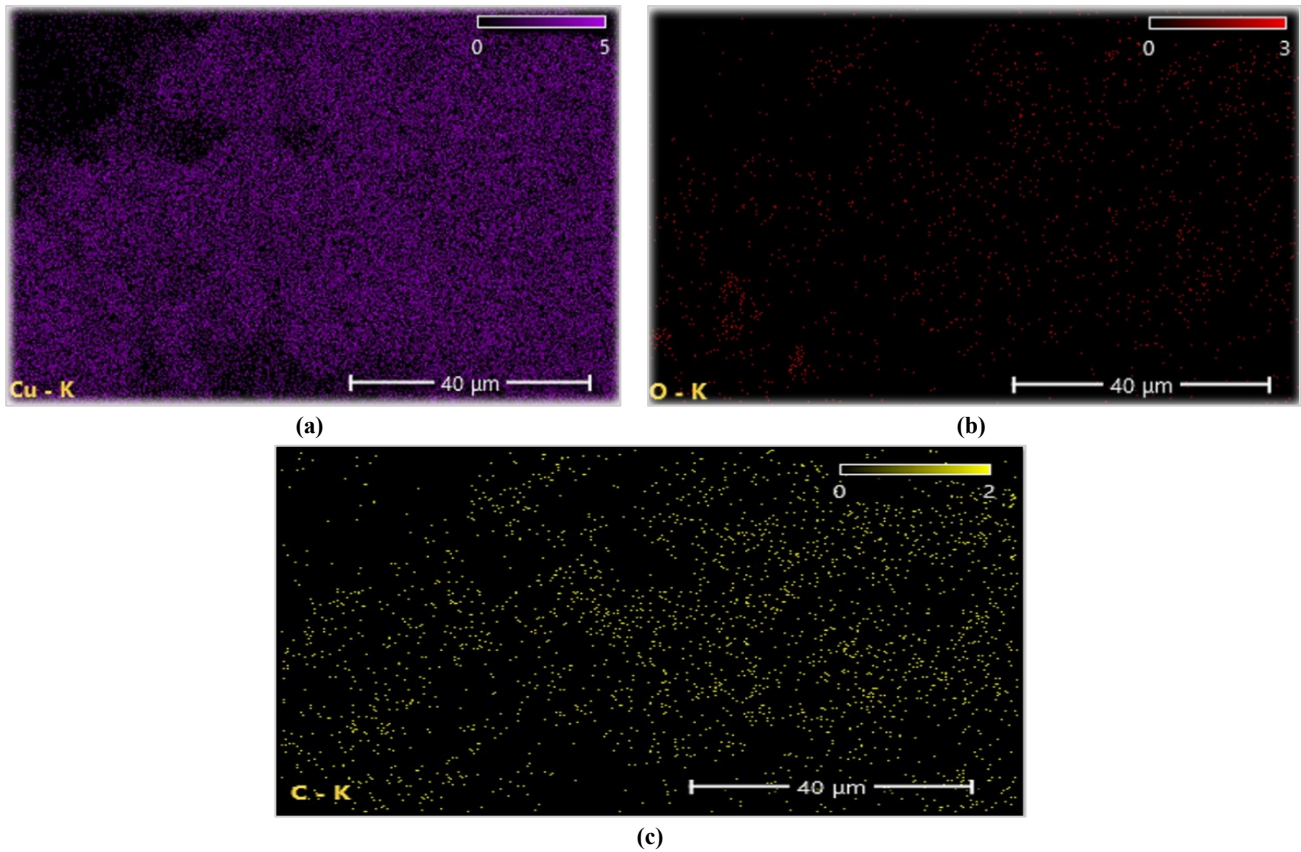


Figure 13: Elemental map elements for: (a) copper produced by the addition of amylase at concentration (0.25, 0.50, 0.75 and 1) g+ 100 g CuSO₄ , (b) weak oxygen and (c) little carbon

4.4 The zeta potential analysis

The zeta potential investigation of the CuNPs indicates the stability of copper nanoparticles. In the current study, observed at +3.00 mV without additive from Figure 14 , it can be seen that the dispersion of the zeta potential of the ideal sample (20 g of CuSO₄) before the addition has an average diameter (3) nm, the size of zeta potential value was (3.84) mV compared to the dispersion that occurred after adding the amylase enzyme with zeta potential (2,- 65, 32 and 3) nm of particles size respectively where we notice that the high charges of both the negative and positive particles lead to the stability of the particles in the liquid, their non-precipitation, and preventing them from agglomeration, the negative charge important indicator for particles size and appears that particles size is smaller than 100 nm as shown in Figure 15 (a, b, c and d). It will tend to repel each other, and there will be no tendency for the particles to come together, which gives long-term stability owing to the electrostatic stabilization of a colloidal particle. The results of zeta potential agreed with Verma and et.al. where explained the negative charge indicates that the particle size is smaller than 100 nm. The negative charge on nanoparticles due to citrate ions is another important indicator of particle size.

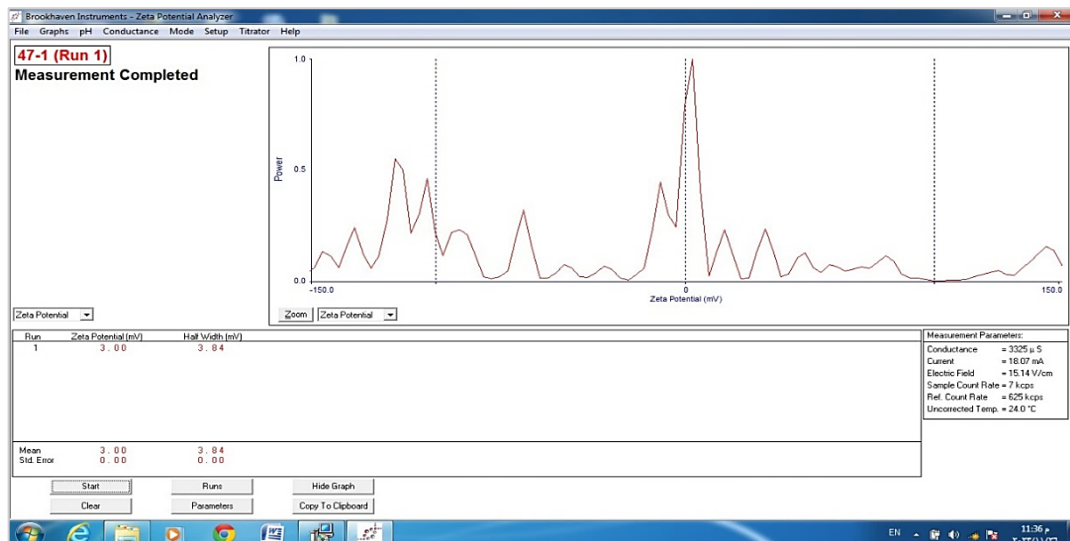
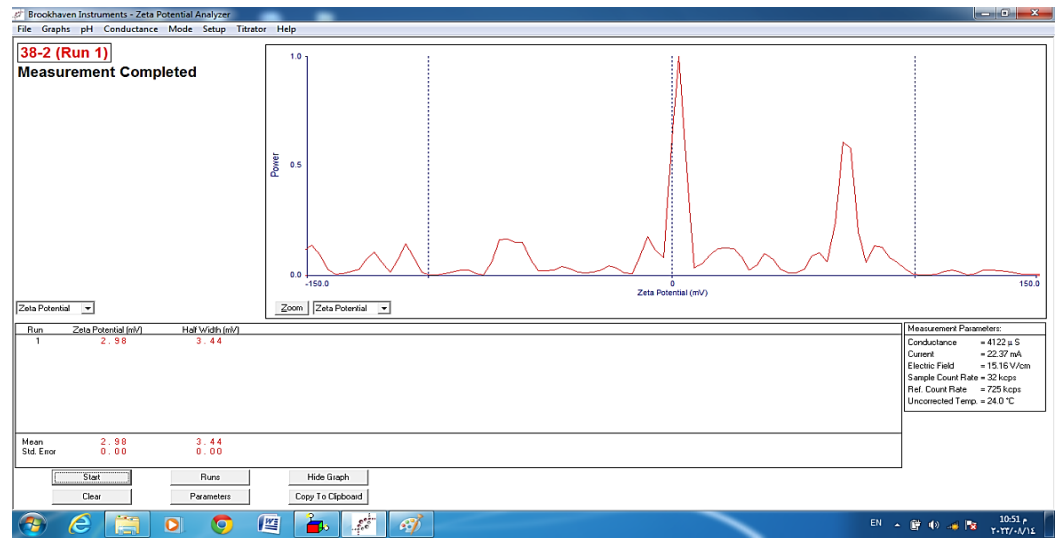
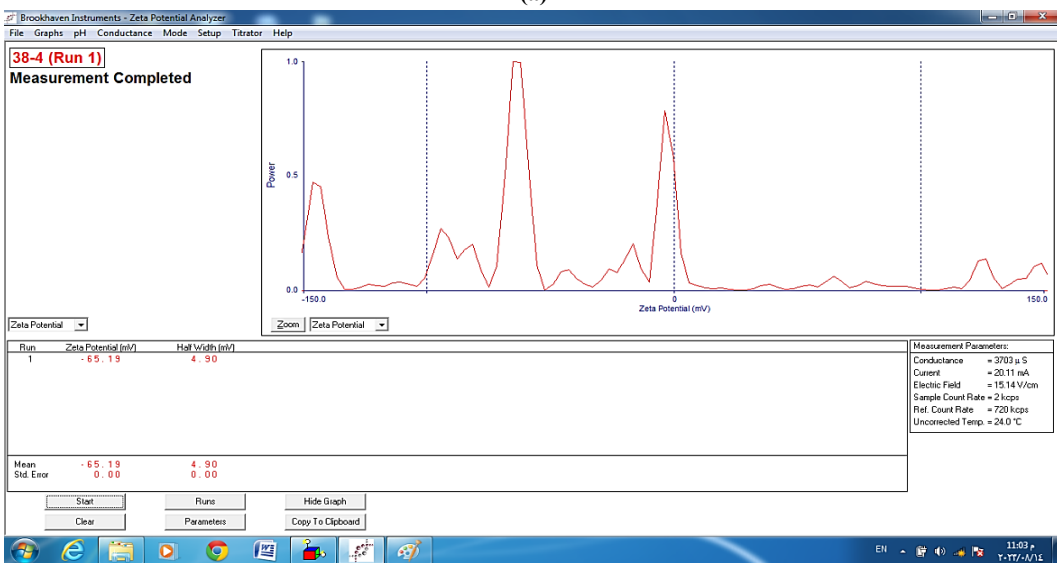


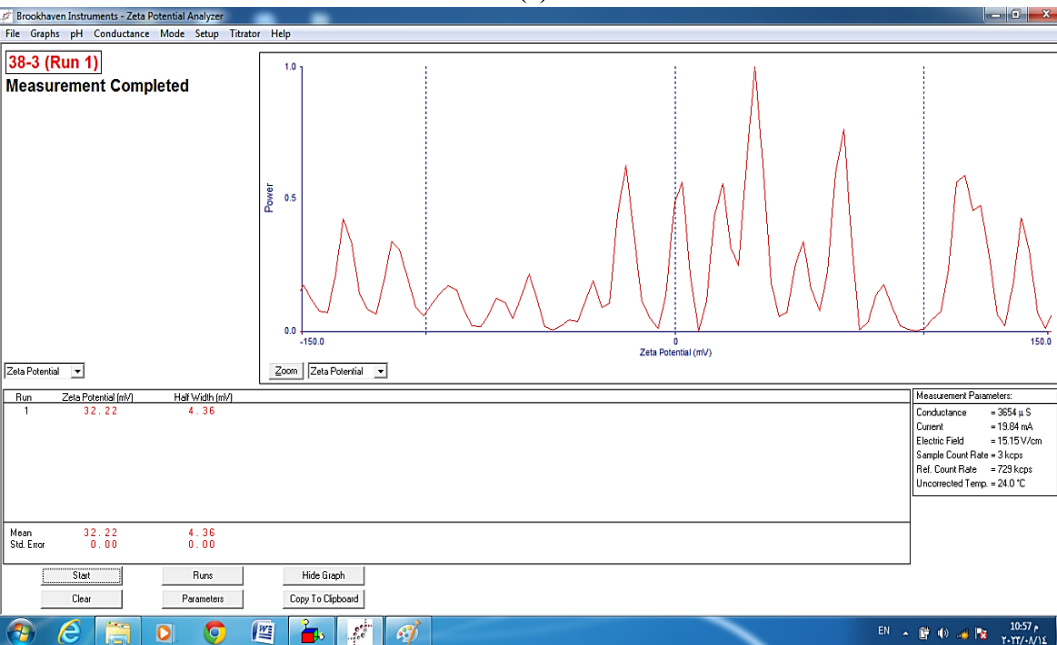
Figure 14: Zeta potential distribution of CuNPs of from 20 g CuSO₄ without additive enzyme amylase



(a)

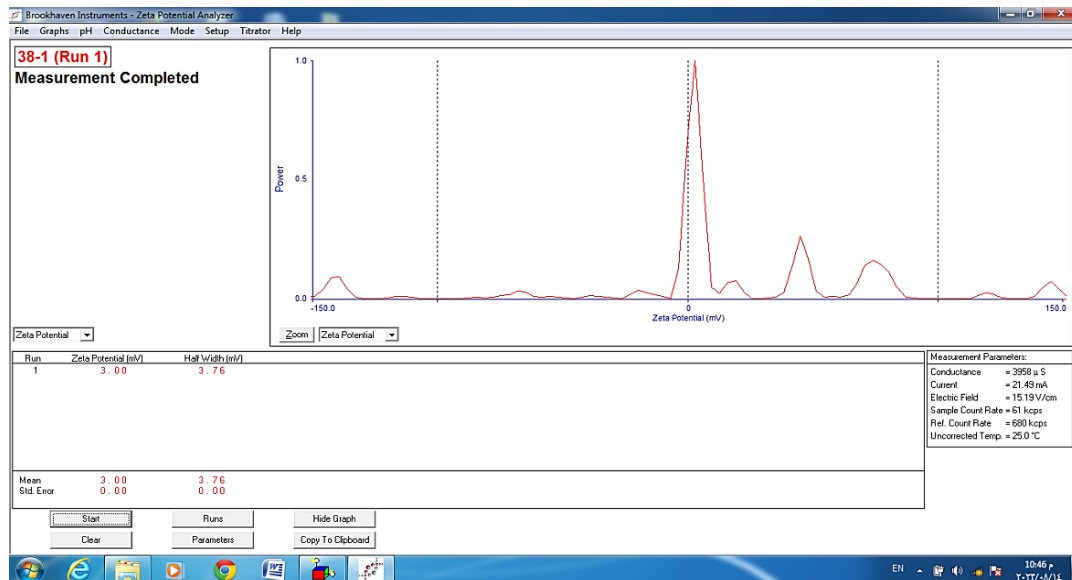


(b)



(c)

Figure 15: The zeta potential distribution of CuNPs of a and b) in 0.25 and 0.50 enzyme additive, and (c and d) in 0.75 and 1 g of enzyme additive at deposition of one hour, 100 g/L CuSO₄ solution



(d)

Figure 15: Continued

5. Conclusion

From the results obtained in this work, it can be concluded that the preparation method of copper nanoparticles (CuNPs) by employing the copper sulphate as precursor material using an electrodeposition process has been successfully developed. Cu Nanoparticles colloid is formed with various sizes and shapes and uses an ideal concentration of CuSO_4 (20 g) with variables concentration of additive (0.25, 0.50, 0.75 and 1) g under condition process. In this work, copper nanoparticles (CuNPs) were effectively synthesized by easy, rapid, non-toxic, and eco-friendly methods via the electrodeposition process utilizing the amylase enzyme. These organic sumps work by creating chelating compounds with the metal ion through coordination bonds, and through this complex compound, we obtain stability and good dispersion of the particles, and the production of copper powder with small sizes and regular shapes. In particular, CuNPs have been characterized using DLS, FE- SEM techniques, EDX analysis, and zeta potential. Specifically, polydisperse and regular nanoparticles of about 44nm were synthesized. As a result, the production of highly efficient copper powder was conducted electrochemically. The increase in the current density of the copper powder deposition led to an increase in the weight of the copper powder deposition at an optimum sample with the 3.13 g copper powder at 0.164 mA/cm² and with the 0.50 g concentration of amylase enzyme.

Author contributions

Conceptualization, F. Sayyid, M. Hafiz, W. Isahak and N. Hadi ; data curation, M. Hafiz; formal analysis, W. Isahak .; F. Sayyid.; project administration, N. Hadi, resources, F. Sayyid.; software, W. Isahak.; supervision, F. Sayyid.; validation, N. Hadi., F. Sayyid. and M. Hafiz.; visualization, F. Sayyid.; writing—original draft preparation, N. Hadi.; writing—review and editing, F. Sayyid. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

Conflicts of interest

The authors declare that there is no conflict of interest.

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