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RESEARCH ARTICLE - MEDICAL TECHNIQUES

A Study into the Electrochemical Behavior of Nano Antibiotics as A Promising Treatment for Helicobacter Pylori Infection by Cyclic Voltammetry

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Abstract

The helicobacter-pylori (H.pylori) bacteria that infects the digestive system is one of the bacteria that is intractable in routine treatments with antibiotics, as it has been studied using nano-antibiotics studied by the electrochemical behavior of three Nano antibiotics.
The study aims to identify the extent of the effect of micro-antibiotics on the composition of the blood serum medium by electrochemical analysis using the cyclic voltammetric method and to compare it with nano-antibiotics.
Three Nano-antibiotics such as Amoxicillin NPs, Azithromycin NPs, and Metronidazole NPs, and the mixture of the three nanoparticles as Nano-Kit were investigated in blood serum medium utilizing cyclic voltammetry methods by a glass carbonic electrode (GCE). The three nanoparticles in antibiotics were prepared by converting the micro antibiotic with a lyophilizer technique and characterization by field emission scanning electron microscopy (FESEM) with all antibiotics in Nano size.
The results showed that all Nano compounds act as an antioxidant antibiotic in the blood serum medium. The study also included the electrochemical characterization of the Nano-kit of the mixture for the three Nano-biotics to prove anti-oxidative stress. It was found that the three nano-biotics have reduced current peaks without any oxidation peaks.
The three nano-antibiotics can be an excellent treatment to eradicate H. pylori infection permanently. Therefore, the results of this research can be adapted to use the nano-kit of the mixture in the appropriate treatment of H.pylori infection.

Publisher : Middle Technical University

Keywords: Amoxicillin NPs; Azithromycin NPs; Cyclic Voltammetry FESEM; H-pylori infection; Metronidazole NPs; Nano-kit; Serum Blood Samples.

1. Introduction

Helicobacter-pylori (H.pylori) is a bacterium whose routine treatments do not eradicate it. The new science now uses nanotechnology to treat these bacteria completely because this technique is highly effective for these bacteria [1-5]. Helicobacter pylori is a persistent organism present in around fifty percent of the world's population. Chronic H. pylori infection results in necrotic alterations, even stomach metaplasia, and is known to be linked to peptic ulcer disease [6]. Peptic ulcer disease, the much more prevalent stomach condition, is now considered a contagious disease, and all present conferences concur that Helicobacter pylori are currently used as a template for persistent bacterial infections that are cancerous [7]. The H. pylori assay was tested using a carbon nano-electrode supported by bismuth..The created sensor can be applied in clinical applications where the peak current of the patient is 100 times greater than that of passive normal tissue [8]. When the diagnostic square wave stripping working range of 0.72-7.92 µg/ml of helical DNA (11 spots) was attained, the potential of the reduction peak emerged at 0.4 V. A sensitive and specific electrochemical approach has been devised to determine amoxicillin using a smear electrode made from naturally occurring carbon nanotubes treated with carboxylic acid. At pH 10.5 phosphate-buffered solution, the modified electrode demonstrated good electron donor activity for the electrochemical oxidation of amoxicillin. With a detection limit of 8.7 nmol L-1 amoxicillin, the electrocatalysts oxidizing peak current of amoxicillin displayed two linear dynamic ranges. Using the voltmeter square wave approach, the linear calibration ranges were between 0.03-0.35 µmmol L and 0.50-32.70 µ mol L amoxicillin. Lastly, the ability of this electrode material to recognize amoxicillin in urine and actual drug specimens was also tested [8].
Nanomaterials with exceptional thermal, mechanical, optical, and electrical capabilities have been highlighted as one of the most promising materials for opening new portals to create next-generation biosensors. This article presents the most recent advances in constructing nanomaterial-based biosensors to detect antibiotics. Current issues and potential future developments in the field are also discussed to create a direction for future study directions [9]. Helicobacter pylori wasn't really harmed by antibiotic tests using metronidazole or gold nanostructures. Some alterations showed that metronidazole had bound to gold nanoparticles. Consequently, the growth inhibition area of the mixture of metronidazole and gold nanoparticles is 17 mm [10]. The bacterial survival rate increased compared to amoxicillin therapy and nanomaterial's heating alone. The protection biofilm brought on by heating can be partly blamed for the synergism, which may make bacteria more susceptible to antibiotics. With the ability to lessen adverse effects and increase the effectiveness of battling drug-resistant strains, our technology offers a

Nomenclature			
H-pylori	Helicobacter pylori	Nm	Nanometer
CV	Cyclic Voltammetry	µg/ml	microgram per mill
AFM	Atomic Force microscope	µmol L	micro moll per litter
NPS	Nanoparticles	$Vs.^{-1}$	reference electrode at a scan rate

practical method for treating H. pylori infection [11]. Clarithromycin, amoxicillin, and proton pump inhibitors eradicate H. pylori. However, a significant part of the population cannot tolerate it. Because of this, a brief course of treatment with these medications is affordable, but their effectiveness in terms of killing the bacteria has not yet been proven [12]. Aim of the study: In this work, the new Nano-antibiotic such as Amoxicillin NPs, Azithromycin NPs, and Metronidazole NPs, and mixed of them have studied the electrochemical effect in serum blood medium and compared with the micro-antibiotics using the cyclic voltammetric technique.

2. Materials and Methods

2.1. Materials

Between October 2021 and February 2022, researchers worked to isolate H. pylori from patients with peptic ulcers or gastritis who had positive IgM test results. Patients consent was acquired prior to sample collection. Isolation of H. pylori from stool samples of such individuals was cultured on Columbia agar supplement with an anaerobic condition with 5%CO2 and sheep RBCs, or sheep serum. Inoculated plates were kept in an aerobic condition with the gas pack for 72 hrs. Three biopsy samples were collected. Confirmative tests included the urease test, catalase test, oxidase test, and PCR. The rapid urease test was done on all samples as soon as they were delivered to the laboratory, using tryptic soy broth as the transport medium under anaerobic conditions. The samples were collected from the (gastroenterology and liver hospital in Baghdad Medical City). Microscopic examination by gram staining was done on all bacterial isolates grown on Columbia agar. The colonial morphology of grown bacteria on Columbia agar was examined to meet the standard morphology of H. pylori colonies of suspected bacteria and appeared as small, white, opaque colonies with no hemolysis. Amoxicillin, azithromycin, and metronidazole powder from Samara Pharmaceuticals (SDI CO., LTD. 8-1 centrifuge type (3000 rpm by dissolving (2.6 g/50 ml DW)-distilled water, the water fluid made with deionized water. Each blood specimen was diluted in deionized water. A circular cell voltmeter was filled with 10 mL of diluted serum that had been ionized in a ratio of 1:9 mL (serum: deionized water). All materials were of 98-99.9% purity and were thus used without any additional purification process[13].

2.2. Apparatus

A cyclic voltmeter (EZST 12051401, NuVant Systems, Inc., and Crown Point, IN, USA) was used to study the electrochemical analysis. A field emission scanning electron microscopy (FESEM) model, MIRA III from (TESCAN, Czech), was used for size measurements for Nanoparticles and morphology of amoxicillin NPs, azithromycin NPs, and metronidazole NPs.

2.3. Preparation of Nanoparticles

A lyophilizer from LABCONCO (USA) was used to prepare both amoxicillin NPs, azithromycin NPs, and metronidazole from fine particles by deep freeze methods.

2.4. Procedure

In this study, the oxidation-reduction reactions of amoxicillin NPs, azithromycin NPs, and metronidazole in plasma level samples were determined using a potentiostat. The working, reference, and counter electrodes were placed in the solvent of the cyclic voltammetric cell, which connected the three electrodes in the potential station, and the probable station was then connected to a personal computer to produce the cyclic voltmeter.

3. Results and Discussion

3.1. The effect of the nanoparticles treatment on H.pylori in blood medium

A new Nano-treatment of H-pylori bacteria was used by three types of nano-antibiotics: amoxicillin, azithromycin, and metronidazole at nanoparticle forms in the blood medium.

3.1.1. Amoxicillin Nanoparticles

Using the cyclic voltammetric method with GCE as the working electrode and Ag/AgCl as the reference electrode, amoxicillin nanomaterials at various concentrations in blood serum media were studied. Fig. 1. The cyclic voltammogram of Fig. 1 shows only the reduction of current peak for amoxicillin nanoparticles in blood serum medium at-400 mV, which is enhanced by increasing the concentrations as shown in Fig. 2 with the relationship between the reduction peak delete to the concentration. So, the amoxicillin nanoparticles act as an anti-oxidative reagent that can be used in treatment for the H. pylori infection instead of using amoxicillin microparticles, which have an oxidation peak at potential of 1.0 V. Fig. 3 illustrates the excellent relationship with the equation:

$$Y=14.818x+21.8 \text{ with high sensitivity of } R2=0.9738$$

Amoxicillin nanoparticle antibiotics act as an anti-oxidant reagent in alkaline blood serum media with only a reduction peak. In contrast, the oxidation peaks have not appeared in the acidic blood serum medium, as shown in Fig. 1. Amoxicillin nanoparticles can be used in alkaline blood serum as a promising treatment as antibiotics for different bacterial diseases such as H. pylori without any side effects [14, 15].

3.1.2. Azithromycin Nanoparticles

By using cyclic voltammetry measurement in blood serum media with the lowering peak at-500 mV, the electrochemical performance of azithromycin nanoparticles was investigated at various doses ranging from 0.02 to 0.2 mM, as shown in Fig. 4. The reduction peak of azithromycin in blood serum medium has been enhanced by increasing the concentration, which has an excellent linear relationship of equation

y=16.394x+22.087 with high sensitivity of R2=0.9135 as shows in Fig. 5.

As a result, azithromycin nanoparticles in blood serum media operate as an antioxidant reagent and, therefore can be utilized as an effective, harmless cure for various infectious infections, particularly for the infection of the stomach bacterium H. pylori. [16]. Fig. 6 illustrates the effect of azithromycin nanoparticles in different pH of blood serum medium, an alkaline pH medium causes enhancement of the reduction current peak without any oxidation reaction, while in an acidic medium two oxidation peaks appear, so the azithromycin nanoparticles are a safety treatment used for *H-pylori* infection [17].

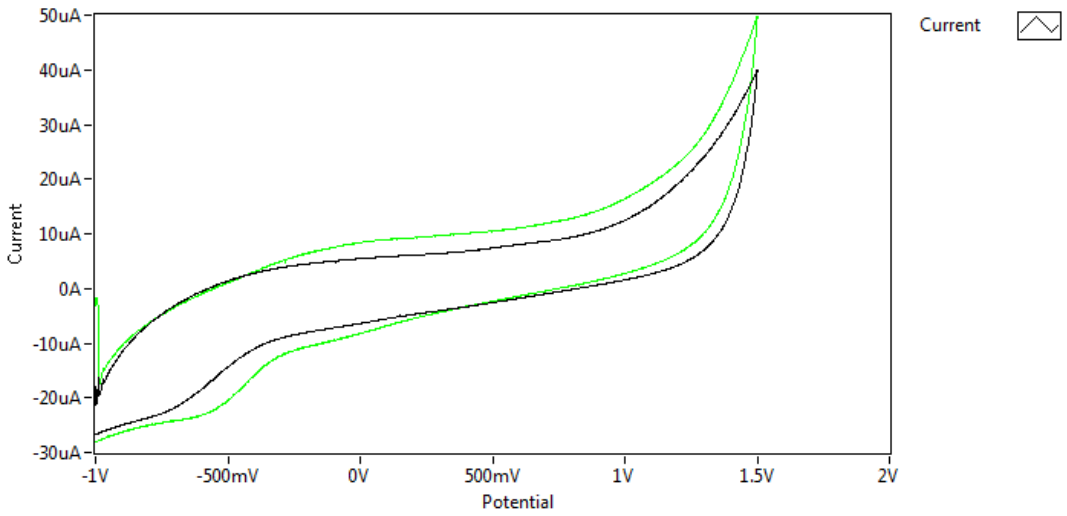


Fig 1. Cyclic voltammetry of amoxicillin nanoparticles at various concentrations in serum blood media on GCE of the working electrode against Ag/AgCl of the reference electrode at a scan rate of 0.1 Vsec-1

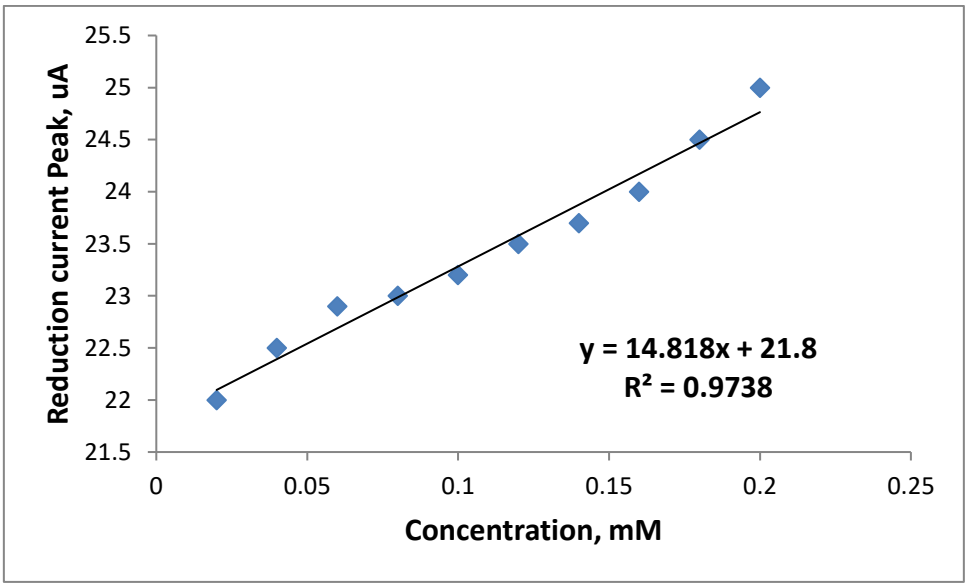


Fig 2. Relationship between reduction current peak against the concentrations (0.02-0.2 mM) of amoxicillin nanoparticles in serum blood medium

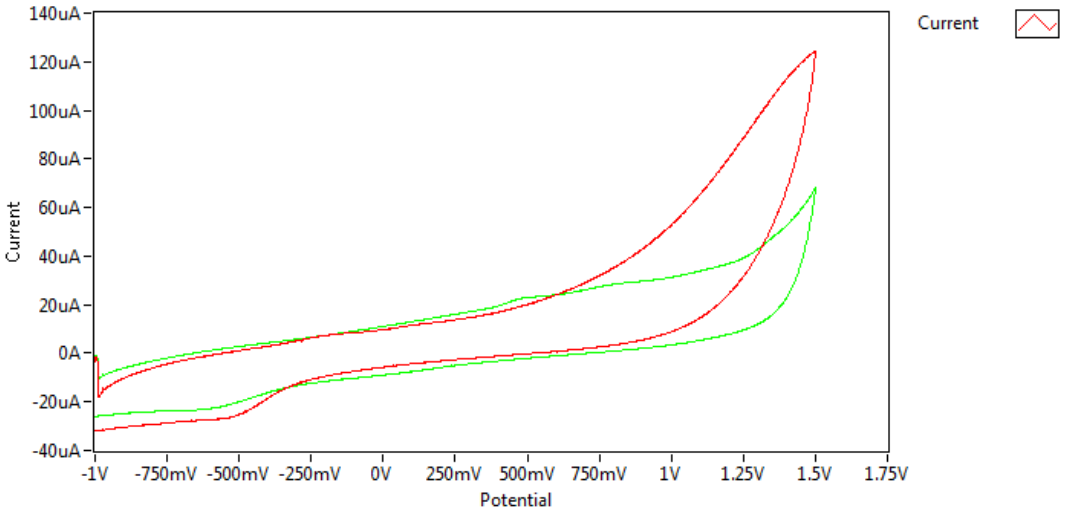


Fig 3. Cyclic voltammetry of amoxicillin nanoparticles at various pH (red line at pH 11 and green line at pH 2) in blood serum media on GCE of the working electrode against Ag/AgCl of the reference electrode at a scanning rate of 0.1 Vsec-1

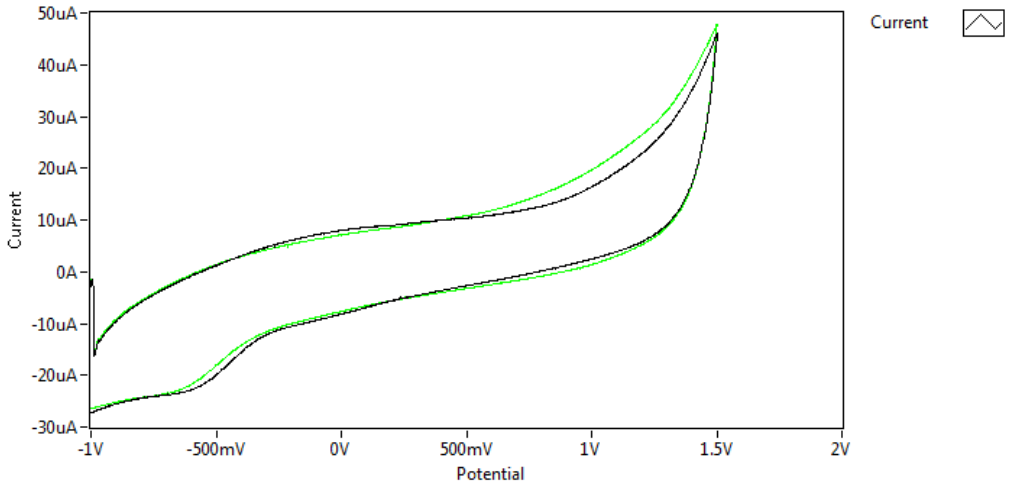


Fig 4. Cyclic voltammetry of azithromycin nanoparticles at various concentrations of serum blood media on GCE of the working electrode against Ag/AgCl of the reference electrode at a scan rate of 0.1 Vsec-1

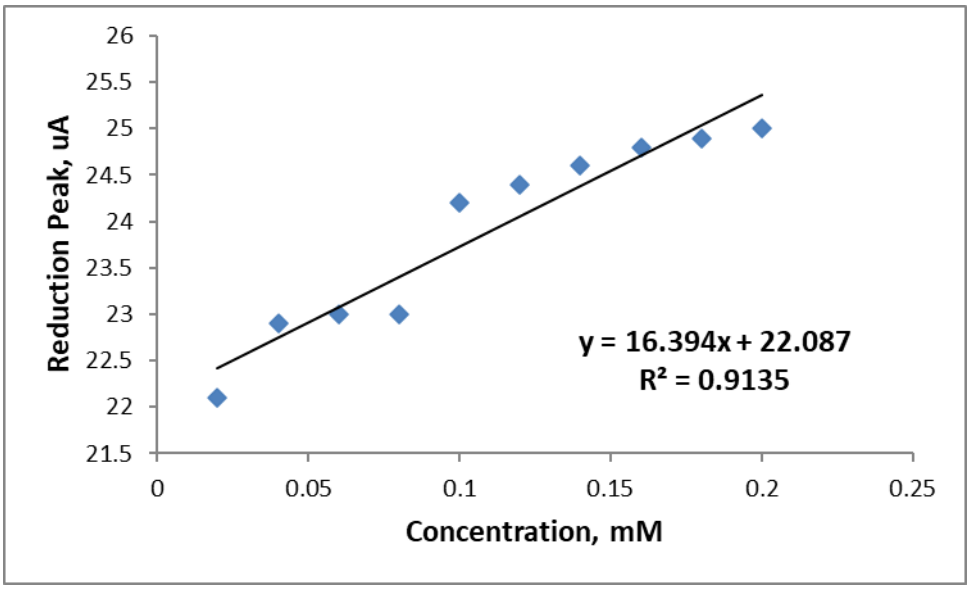


Fig 5. Relationship between reducing current peak toward the concentrations (0.02-0.2 mM) of azithromycin nanoparticles in blood serum media

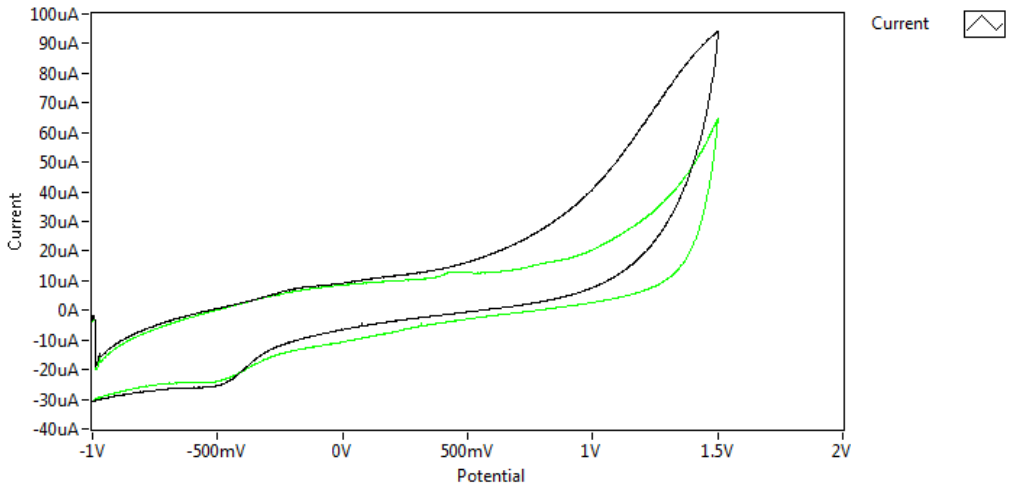


Fig 6. Cyclic voltammogram of azithromycin nanoparticles at different pH (black line at pH 11 and green line at pH 2) in serum blood medium on GCE as working electrode against Ag/AgCl of the reference electrode at a scanning rate of 0.1 Vsec-1

3.1.3. Metronidazole NPs

Metronidazole NPs solution has different behavior than each of amoxicillin NPs and azithromycin NPs in blood serum medium that shows two reduction peaks without any oxidation peak, so metronidazole NPs is considered a strong, active antibiotic against different bacteria, especially of *H. pylori* as shown in Fig. 7. In different concentrations of metronidazole NPs, the two reduction peaks are enhanced through the increase in the concentration, which has a strong linear association with different concentrations from 0.02 to 0.2 mM as shown in Fig. 8. The equation of the relationship is:

$$y = 50.636x+28.98 \text{ with a high sensitivity of } R2 = 0.9395$$

Fig. 9 illustrates the cyclic voltammogram of metronidazole nanoparticles at various pH (the line black in pH two and the line green in pH 8) in blood serum medium; the electrochemical behavior of metronidazole NPs in acidic serum medium at pH 2 has two oxidation peaks and a reduction peak. so, the acidic medium causes the antibiotic to become oxidative stress, while in alkaline serum medium it acts as an antioxidant reagent with two reduction peaks.

3.2. Mixture of Amoxicillin NPs, Azithromycin NPs, and Metronidazole NPs (Nano-Kit)

In this study, a Nano-kit of three Nano-antibiotics, consisting of a mixture of amoxicillin NPs, azithromycin NPs, and metronidazole NPs, was used to study their electrochemical behavior in the blood serum. Fig. 10 illustrates that the cyclic voltammetry of the Nano-kit in blood serum has one to two reduction peaks without any oxidation peak, so it can be said that the Nano-kit has perfect results in different concentrations with the good linear relationship of the equation:

$$y=63.285x+21.435 \text{ and the high sensitivity of } R2 = 0.8583 \text{ as seen in Fig. 11.}$$

The cyclic voltammetry of the Nano-kit of mixture Nano antibiotics in blood serum medium at different pHs is shown in Fig. 12. In an alkaline medium, two reduction peaks appeared without any oxidation peaks, so the Nano-kit can be depended on to be used as a treatment for difficult bacteria such as H. pylori infection. While in acidic pH, the Nano-kit acts as an oxidant stress because the oxidant peak appears in the cyclic voltammetry. The use of nanoantibodies in other work or local work has been observed in various medical fields without their use in electrochemical studies [16, 18-20].

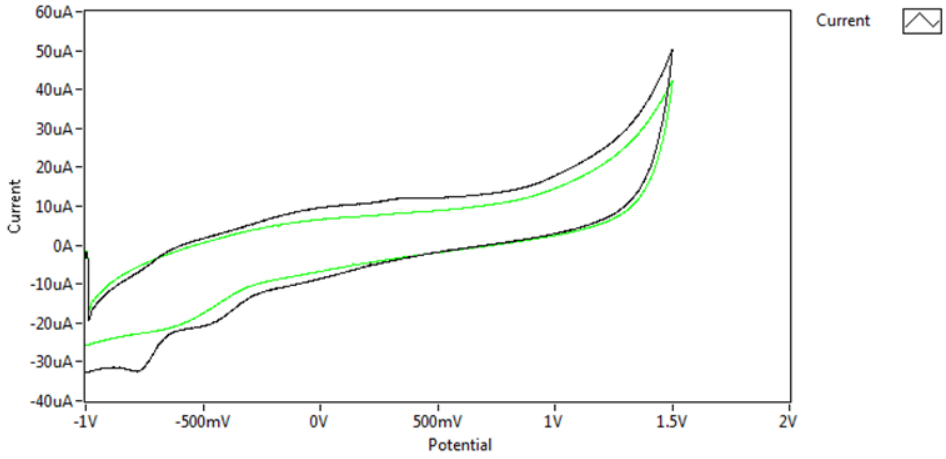


Fig 7. Cyclic voltammetry of metronidazole nanoparticles in various concentrations at blood serum media on GCE of the working electrode against Ag/AgCl of the reference electrode at a scanning rate of 0.1 Vsec-1

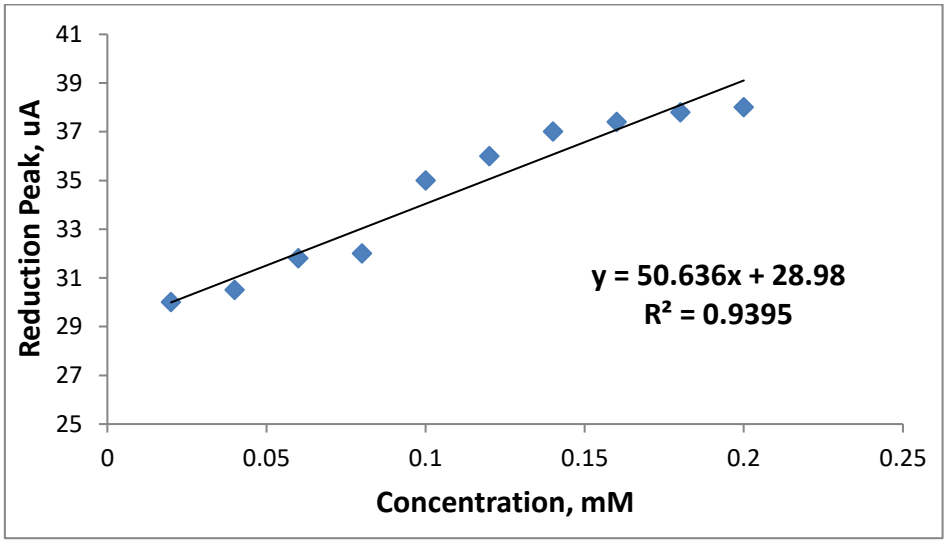


Fig 8. Relationship between reducing current peak as opposed with concentration (0.02-0.2 mM) of metronidazole nanoparticles in blood serum media

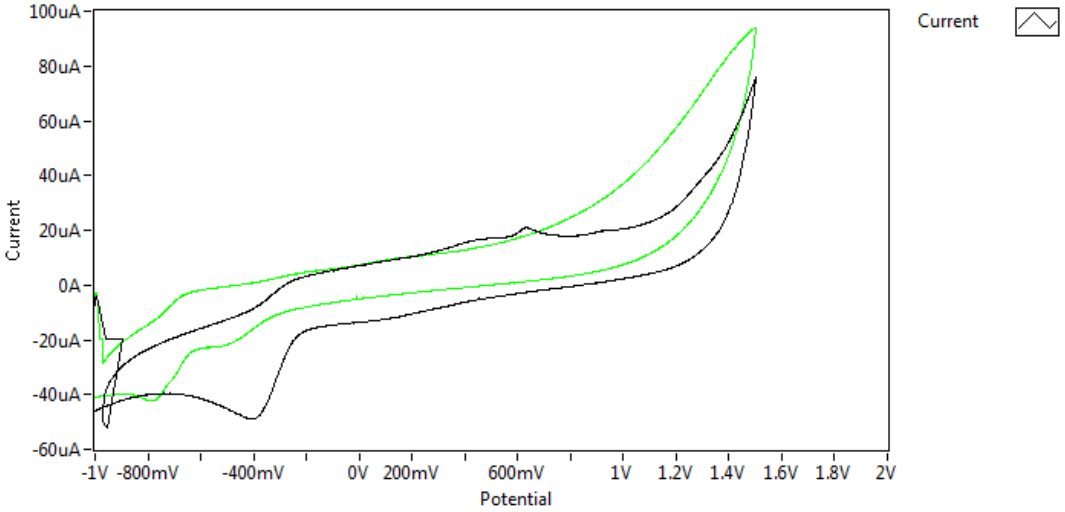


Fig 9. Cyclic voltammogram of metronidazole nanoparticles at different pH (black line at pH two and green line at pH 8) in serum blood medium on GCE of the working electrode against Ag/AgCl of the reference electrode in scanning rate of 0.1 $Vsec^{-1}$

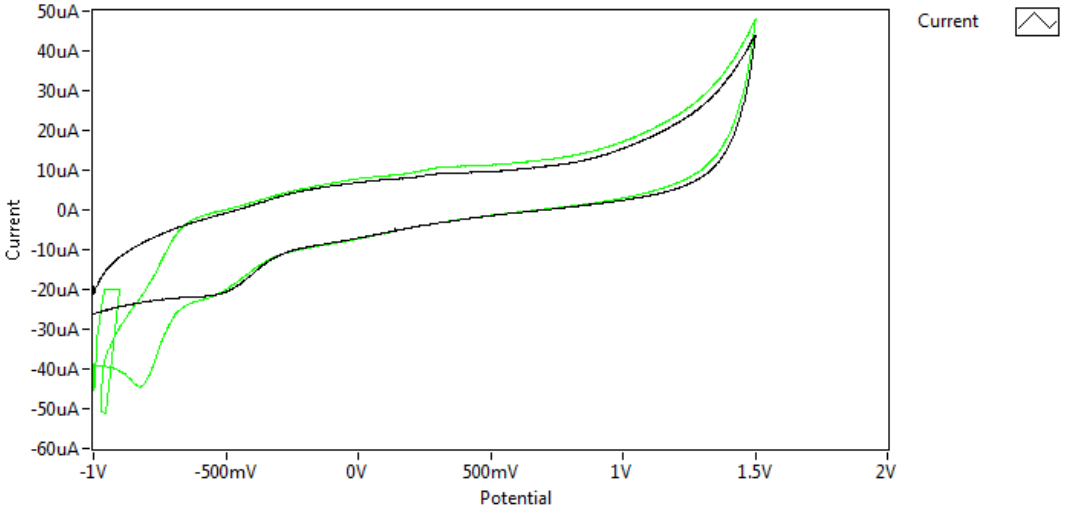


Fig 10. Cyclic voltammogram of the mixture of amoxicillin NPs, azithromycin NPs, and metronidazole NPs at different concentrations of 0.02-0.2 mM at blood serum media on GCE of the working electrode against Ag/AgCl of the reference electrode at a scanning rate of 0.1 $Vsec^{-1}$

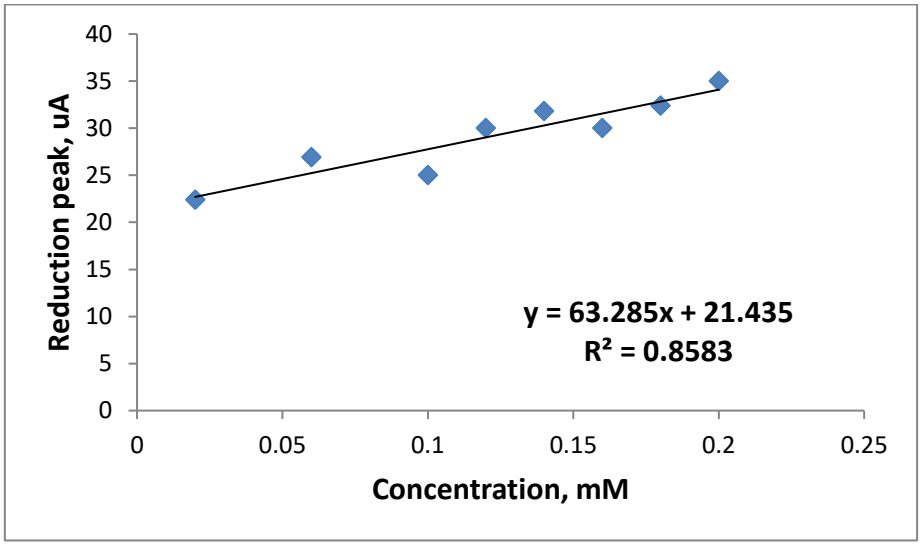


Fig 11. Relationship between reducing current peak towards the concentrations (0.02-0.2 mM) of amoxicillin NPs, azithromycin NPs, and metronidazole NPs in serum blood media

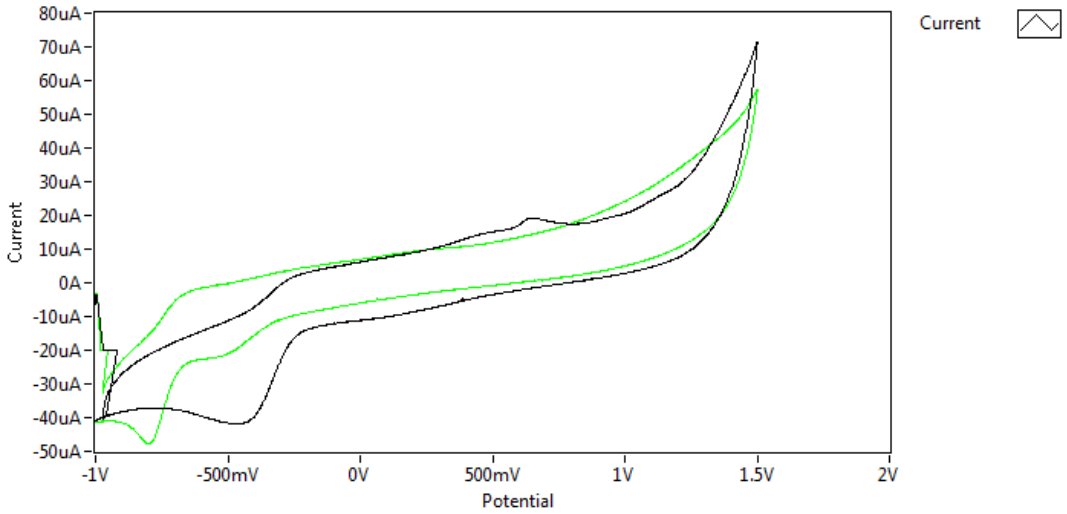


Fig. 12. Cyclic voltammogram of a mixture of amoxicillin NPs, azithromycin NPs, and metronidazole NPs in various pH (black line at pH two and green line at pH 8) at blood serum media at GCE of the working electrode against Ag/AgCl of the reference electrode by scanning rate of 0.1 $Vsec^{-1}$

3.3. Reliability and Stability Study

An electrochemical method can be used in evaluating the operation of the cyclic voltammeter by obtaining a ten-times scanning of the cyclic voltammogram to ensure the accuracy of the work with this technique (CV). Fig. 13 illustrates the cyclic voltammogram of the mixture of the three Nano-antibiotics in Nano-kit in blood serum media. It has a high degree of crossover in the voltammetric research and good results stability of the lines of the cyclic voltammetric, which confirms the reliability of scanning. It was found the RSD of the reduction current peak of the Nano-kit mixture had a good value of ±1.45.

3.4. Field emission scanning electron microscopy (FESEM) study

In this research work, the FESEM machine was used to examine the sample's surface topography and composition. Fig. 14 shows the FESEM for the (a)amoxicillin NPs, (b) azithromycin NPs, and (c) metronidazole NPs which have the morphology and the dimension of nanoparticles of the three nano-antibiotics.

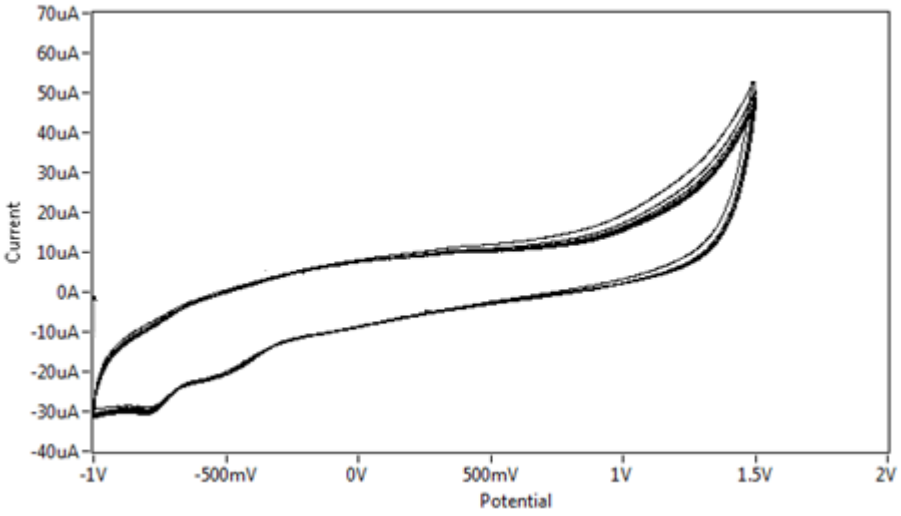
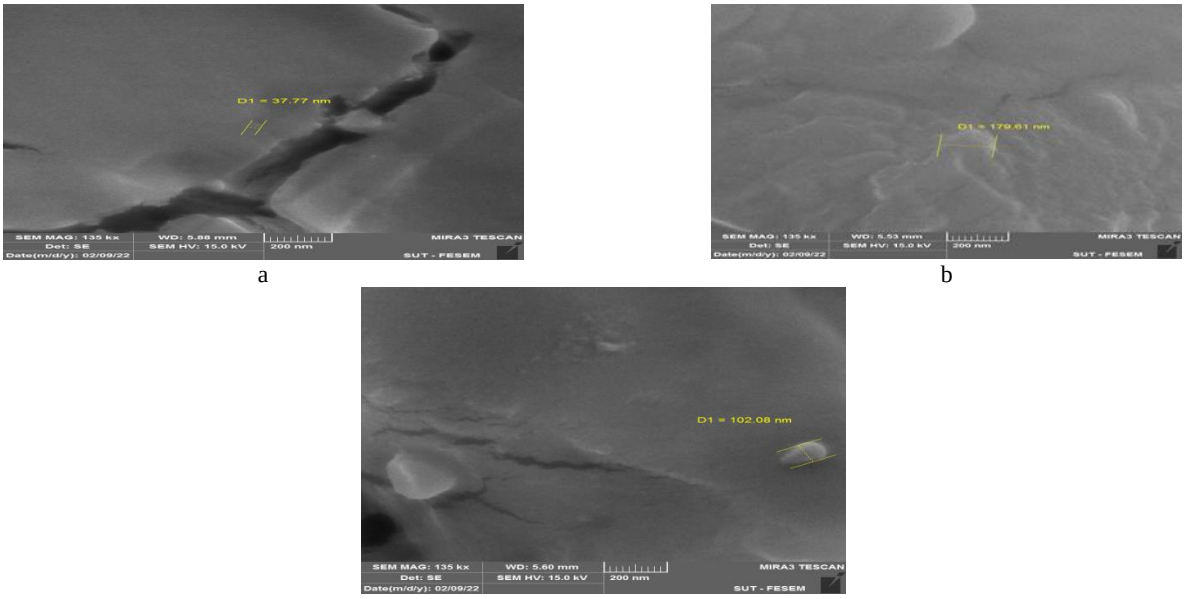


Fig 13. Cyclic voltammogram of ten times scanning for the Nano-kit in serum blood medium



a b c

Fig 14. FESEM for the (a) amoxicillin NPs, (b) azithromycin NPs, and (c) metronidazole NPs

4. Conclusion

The nanoparticles of the three antibiotics of Nano-kit (a mixture of amoxicillin NPs, azithromycin NPs, and metronidazole NPs) were produced by deep-freezing (Lyophilization) antibiotics. The deep-freezing method proved to be a more reliable method for producing nanoparticles with antibacterial effects. The nanoparticles of the three Nano-antibiotics with the Nano dimension were characterized using FESEM. The Cyclic voltammetry (CV) study showed that a new form of the three nano antibiotics in Nano-kit (mixture of amoxicillin NPs, azithromycin NPs, and metronidazole NPS) is safe to be used in the human body since it acts as an anti-oxidative reagent which has all the nanoparticles of the three antibiotics and the mixture of them without any oxidation peaks.

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