RIFAMPICIN NPs: SYNTHESIS AND ELECTROCHEMICAL CHARACTERIZATION IN BLOOD SERUM MEDIUM USING CYCLIC VOLTAMMETRY

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ABSTRACT

The rifampicin (RF) was converted to the nanoparticles (NPs) using the lyophilization technique, and the morphology and dimension of the RF NPs were identified by atomic force microscopy (AFM) and field emission scanning electron microscopy (FESEM), which confirm the nano dimension of RF NPs. The importance of the RF NPs as a wide spectrum of antibiotic was identified in this study on its behavior in the serum medium using cyclic voltammetric technique through the redox current peaks in different studies such as the concentrations, pH, scan rates and reliability. It was found from the results that RF NPs in blood serum medium has a single reduction current peak at -500 mV, which can be used RF NPs as good antioxidant antibiotic and a safety treatment for human. Through the study of the reduction current peak of RF NPs, it showed a high-precision in electrochemical properties as treatment of various diseases that outperform the properties of the anti-microbe, especially its effectiveness in the acidic pH = 2 which enhance the reduction current peak of RF NPs.

Keywords: *rifampicin NPs, cyclic voltammetry, blood serum, pH, scan rates.*

INTRODUCTION

The current studies in the field of antibiotics have focused on the use of nano-antibiotic because of the importance of their effectiveness against to the different diseases and the safety of their use on the composition of the blood medium [1 - 5].

Rifampicin (RIF) has a heterocyclic structure containing naphthoquinone, which gives it its characteristic red color with chemical naming of 3,4-methylpiperazine-aliminomethyl rifamycin as shown in Fig. 1 [6]. RIF is synthesized from the derivative of rifamycin, which can be treated in different bacterial infections of tuberculosis, leprosy and other diseases [7].

In the field of cyclic voltammetry the modified electrode has been used in the analysis of RIF in the periphery of the blood due to its large electrically effective surface area and excellent electron transfer ability. Whereas, the manufacture of Ag NPs/3D rGO as a modified working electrode which is used in the study of RIF in human blood samples and drugs. The sensor was used in the characteristic detection of RIF have shown great application in clinical diagnostics, pharmaceutical and nutritional fields [8].

Another study was used of a modified glassy carbon

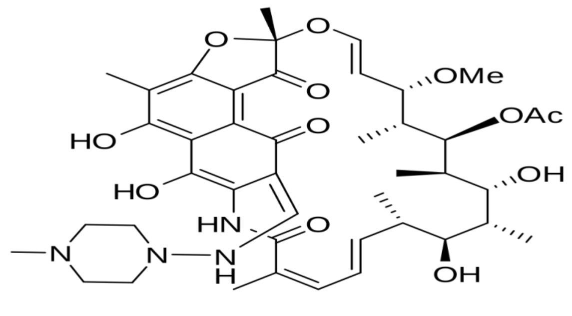

Fig. 1. Structure of rifampicin.

electrode with carbon nanotubes to identify the behavior of RIF in the blood medium which has oxidation and reduction current peaks at 0.5 and -0.5 V, respectively [9].

In a study is using modified glassy carbon electrode with MWCNT on differential pulse and square wave voltammetry of RIF in blood medium. These different techniques have provided selective, rapid, sensitive, accurate and cheap determination of RIF as alternative techniques for liquid chromatography and spectrometric methods in monitoring of therapeutic drugs [10].

The simultaneous use of the nanosystem and the antioxidant can be therapeutically beneficial. Therefore, the simultaneous effects of ascorbic acid and PENs on the release profile and degradation of rifampicin were evaluated, with results confirming its synergistic protective effect at pH 8.5, versus pH 7.4. The benefits of developing nanoparticles in the presence of antioxidants, at an alkaline pH, as an effective approach to reduce rifampicin degradation [11].

In this study RIF NPs were diagnosed as a nano-antibiotic using in cyclic voltammetric technique.

EXPERIMENTAL

Reagents and Chemicals

RF was received from Ajanta Pharma Limited (India), and healthy human blood samples were obtained from the Iraqi Blood Bank in Baghdad Medical City, Baghdad - Iraq, 0.1 N of HCl and 0.1 N of NaOH.

Tools

Adapted by NuVant Systems Inc. Pioneering Electrochemical Technologies, USA, in this study was used to evaluate the electrochemical properties of rifampicin in vitro using an EZstat (potentiostat/glvanostat) series device. In order to output the cyclic voltammogram test, the electrochemical workstations of the integrated analytical system are connected to a personal computer with a potentistat operated by electrolysis software. In addition is using Ag/AgCl (3 M KCl) to calculate the reference while using Platinum wire (1 mm diameter) as a counter electrode. The three electrodes are inundated in a cyclic voltammetic cell (15 mL) with blood diluted with distilled water in ratio of 1:9 mL using as an electrolyte, and using 0.1 M RIF NPs spiking with micro pipette in to the blood medium gradually.

Preparation of RIF NPs

Lyophilization Technique

The product cools, and ice crystals are formed from pure water at -18°C. The second step involves the sublimation of ice from the frozen product by passing convection air from the lyophilizer rack to the frozen solution in the flask, the sublimated ice and water vapor formed pass through the dried part of the product to the sample surface, the water vapor is transferred from the product surface through the chamber to the condenser. The water vapor condenses on the condenser. At the end of the sublimation step, a porous plug is formed. Its pores correspond to the spaces occupied by ice crystals. The third step is drying which involves removing the absorbed water from the product. All steps should be continuous about 48 - 72 hours. Using a lyophilizer, Labconco Company (USA) [12].

Characterization of RF NPs solution

Atomic Force Microscopy (AFM)

Fig. 2 is the AFM of the prepared RF NPs in 17.66 nm which illustrated the morphology details of the nanoparticles as spherical forms [13].

Field Emission Scanning Electron Microscopy (FESEM)

Field emission scanning electron microscopy (FESEM) produces images of a sample by scanning the surface with a focused beam of electrons detailing the shape of nanoparticles as a systematic matrices shapes as acceptable results of rifampicin conversion for micro-particles. According to the structure properties of the nanoparticles, it seems that the dimension of particles included with nano range Fig. 3 shows the FESEM images of RF NPs with the details of the shape of the nanoparticles.

Procedure

Glassy carbon electrode (GCE) was used after cleaning with alumina solution and treated with ultrasonic pathway water for 10 minutes. The three electrodes (reference electrode, Ag/AgCl) at 3M KCl, platinum wire auxiliary electrode with a diameter of 1 mm and glassy carbon electrode as working electrode) were immersed in a cyclic voltammetic cell (15 mL)

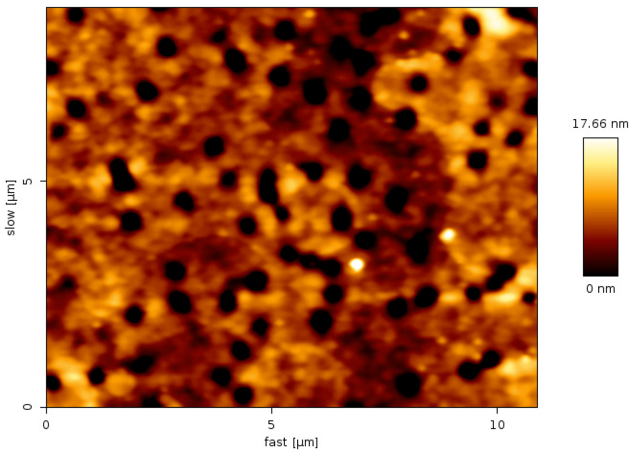

Fig. 2. AFM of RIF NPs.

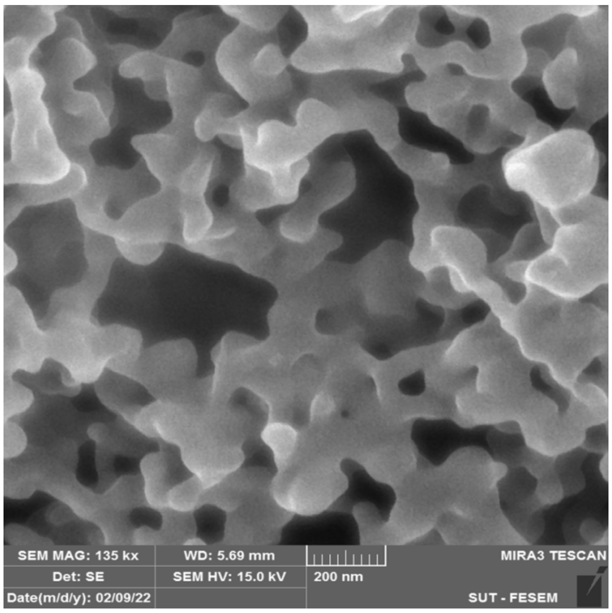

Fig. 3. FESEM of RF NPs.

contain (1 mL of blood serum:9 mL of distilled water). All three electrodes were connected with the potentiostat and with personal computer [14].

RESULTS AND DISCUSSION

Calibration curve

The results of the study showed that RF NPs in the blood serum have an upward effect on the electrical current of the reduction peak because the nanoparticles of RIF act as a good antioxidant reagent. Fig. 4 shows the cyclic voltammogram of RIF NPs at different concentration in blood serum medium. The calibration curve of the relationship between the reduction current peak with different concentraion of RIF NPs from 0.01 mM to 0.06 mM is shown in Fig. 5 as a good linear of the relationship by equation and high value of sensetivity:

$Y = 126.36\ X + 35.286$

$R^2 = 0.9429$

Study at different scan rates

In a cyclic voltmeter (CV), the electrode potential slopes linearly versus time in the cyclic phases. The rate of voltage change over time during each of these phases is known as the trial scan rate ($V\ s^{-1}$). If the electron transfer on the surface of the working electrode is fast and the current is restricted by the diffusion of the analyses to the electrode surface, the peak current will be proportional to the square root of the scan rate. This relationship is described in the Randles–Sevcik equation. In this case, the CV experiments only with a small portion of the solution, i.e. the diffusion layer on the electrode surface [15].

Fig. 6 illustrates the cyclic voltammogram of RIF NPs in serum blood medium at different scan rates (0.01 - 0.1 V sec^{-1}), which has an enhancement of the reduction current peak against to increasing of scan rates as shown in the relationship at Fig. 7 that has a linear line with equation of:

$Y = 292.12X + 8.1333$

$R^2 = 0.9729$

Study at different pH

The voltammetric response of RF NPs has been discussed in relation to the different pH on the GCE that combine both in acidic and alkaline media. Usually, buffer solutions are used to check pH sensitivity, while the effect of ionic strength - rarely [16].

Fig. 8 shows the cyclic voltammogram of RF NPs in serum blood medium at different pH, it was found that alkaline pH enhanced the reduction peak of RF NPs in the serum as illustrated in Table 1 and Fig. 9. While, the acidic pH causes dropping the reduction current peak to lower value, so RF NPs acts as a good antibiotic in alkaline pH.

Study of the Reliability and Stability

It can be evaluated the reliability and stability in cyclic voltammetric study instead of the statistical calculation by scanning the cyclic voltammogram about ten times which indicated the stability of results

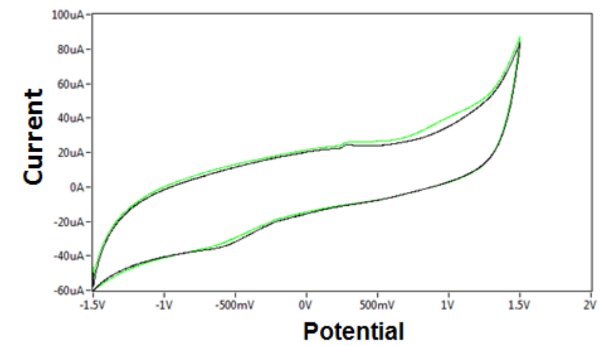

Fig. 4. Cyclic voltammogram of RF NPs at different concentrations in blood serum medium on GCE as working electrode versus Ag/AgCl as reference electrode at scan rate of 0.1 V sec^{-1}.

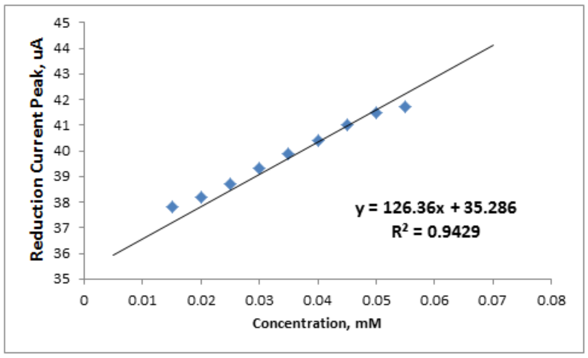

Fig. 5. Relationship between reduction current peak of RF NPs against to different concentrations.

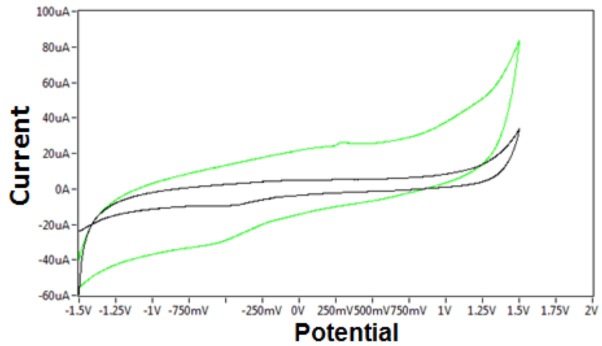

Fig. 6. Cyclic voltammogram of RIF NPs at different scan rates (0.01- 0.1 V sec^{-1}) in blood serum medium on GCE as working electrode versus Ag/AgCl as reference electrode.

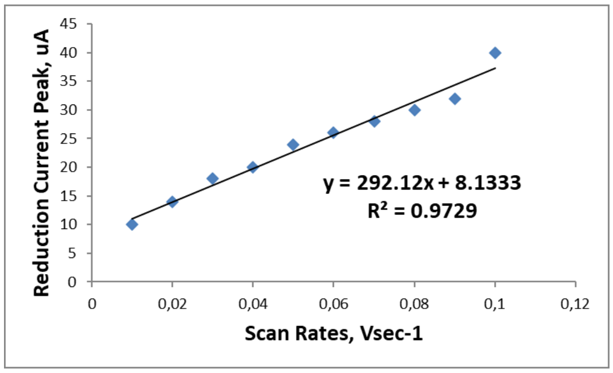

Fig. 7. Relationship between reduction current peak of RIF NPs against to different scan rates (0.01 - 0.1 V sec^{-1}).

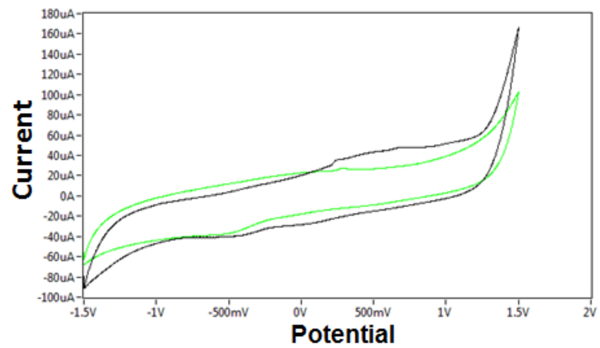

Fig. 8. Cyclic voltammogram of RF NPs at different pH (green line pH = 2 and black line pH = 9) in blood serum medium on GCE as working electrode versus Ag/AgCl as reference electrode at scan rate 0.1 V sec^{-1}.

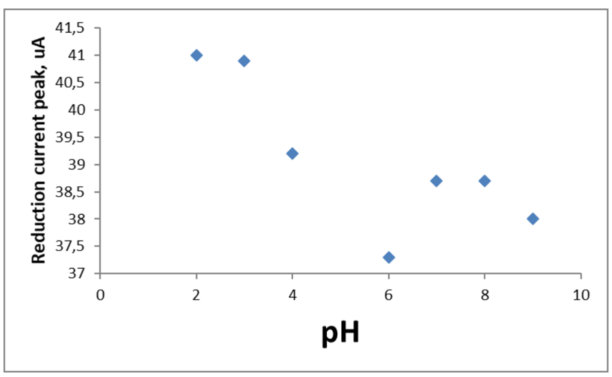

Fig. 9. Effect of pH (2 - 9) on the reduction current peak of RIF NPs in blood serum medium.

Table 1. Effect of pH on the reduction current peak of RF NPs in blood serum.

pH	Ipc
2	-41
3	-40.9
4	-39.2
6	-37.3
7	-38.7
8	-38.7
9	-38

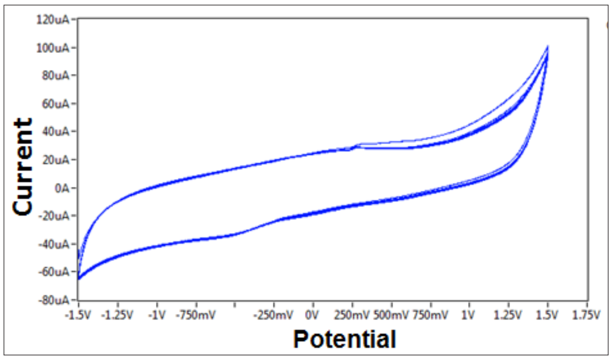

Fig. 10. Cyclic voltammogram of RF NPs at ten scanning in serum medium on GCE as working electrode versus Ag/AgCl as reference electrode at scan rate 0.1 V sec^{-1}.

when overlapping as shown in Fig. 10, and the relative standard deviation (RSD) of the ten scanning at low values. In this work, the RSD of reduction current peak has ±0.5 included with high accuracy in the study of RF NPs in serum blood medium.

CONCLUSIONS

It can be concluded from this study that RF NPs has a good electrochemical property in cyclic voltammetry with the appearance of only one reduction peak and there are no oxidation peaks in the blood serum medium, so it is a safety antibiotic to be used in the treatment of different diseases as an alternative to micro-rifampicin because it is anti-oxidant antibiotic. Moreover, the study illustrated that acidic blood serum medium has been enhanced the reduction current peak and disappeared the oxidation peak, so, it can be used RF NPs in acidic media to working in stomach with high activity against to the different diseases.

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