ELECTROCHEMICAL DETECTION OF DEXTROSE IN ARTIFICIAL SALIVA USING NICKEL OXIDE (NIO)/DIRECT BLUE 71 (DB71) NANOFLOWERS (NFS) ON GLASSY CARBON ELECTRODE (GCE) - A PRELIMINARY STUDY

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Abstract:

Aim:
The aim of the study is to detect Dextrose, which is a simple sugar made from starch. Starch is a naturally occurring complex carbohydrate found in many plants, including corn. In this research, electrochemical detection of dextrose was done from an artificial saliva sample.

Materials and methods:
Electrochemical detection of dextrose was done from an artificial saliva sample. 0.1 M (2.5 g) Nickel acetate tetrahydrate (C4H14NiO8) and 0.2 M (0.8 M) Sodium hydroxide (NaOH) was dissolved in 100 ml of double distilled water (DD H2O). Co-precipitation method was used. After calcination, the sample was again ground well and stored in a 2 ml vial for further
characterization and experiment. 0.1 mM (1 mg) of Direct blue 71 (DB71) was dissolved with 10 ml of DD H2O. Then the dye solution was sonicated in a bath sonicator for 5 mins. 2 mg of NiO was taken and dissolved in a 2 ml of DD H2O and sonicated for 5 mins. Later, 1 ml of DB71 and 1 ml of NiO soln. was mixed in a 2 ml vial and sonicated for 5 mins.

Results:
Field Emission Scanning Electron Microscopy (FESEM) was used for the modified setup to study the morphology of the materials. Ultra violet (UV) Visible spectroscopy was used to find the wavelength with relation to the absorbance of the sample and to confirm the theoretical assumptions experimentally. From data a graph was plotted for different concentration, and calibrated all the concentration data with respect to the resulting current sensitivity.

Conclusion:
The use of Nickel Oxide (Nio)/Direct Blue 71 (DB71) Nanoflowers (Nfs) On Glassy Carbon Electrode (GCE) can be used as a method to detect dextrose in artificial saliva.

Keywords: Saliva, Nanoflower, Dextrose, Biosensors

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1. Introduction:

Wearable sensors have garnered considerable interest over the past decades owing to their broad application prospects and huge development potential. Saliva is increasingly recognised as an attractive diagnostic fluid. The presence of various disease signaling salivary biomarkers that accurately reflect normal and disease states in humans and the sampling benefits compared to blood sampling are some of the reasons for this recognition. Saliva sampling is relatively simple and the presence of various disease-signaling biomarkers in saliva has meant that it can accurately reflect normal and disease states in humans (1). Although saliva collection and determination present some disadvantages, it has been recognised as an attractive diagnostic fluid with an increasing amount of assay developments and technological advancements for the detection of various salivary biomarkers (2). In humans, oral fluid originates mainly from three pairs of major salivary glands (parotid, sublingual, and submandibular) and a large number of minor salivary glands. It also contains fluids from non glandular origin such as oropharyngeal mucosae, crevicular fluid, blood-derived compounds, and food debris (3). Typically, the collection and evaluation of secretions from individual salivary glands are used for the detection of gland-specific pathology such as infection and obstruction. However, due to its easy sampling method, with or without stimulations, whole saliva is more frequently studied especially for the evaluation of systemic disorders (4). Generally, saliva sampling involves a simple and noninvasive collection method that allows easy storage and transport (5). Unlike blood specimens, saliva sampling does not require specialized instruments or trained personnel with phlebotomy skills, it has minimal or no risk of cross contamination among patients and offers very low exposure of healthcare personnel to blood-borne pathogens such as HIV and hepatitis (6). The oral cavity could be a promising body part for getting a variety of health information from gum, teeth, and saliva. The first oral status monitoring device on a partial denture was conducted by Graf in the 1960s to monitor pH and fluoride ion levels (7). Many studies have been focused on the COVID-19 diagnosis method using noninvasive saliva that can be used instead of the examination method utilizing invasive blood collection, which requires medical personnel. As a part of the development of these diagnostic methods, point-of-care (PoC) has been developed to immediately monitor the presence of COVID-19 infection through biomarkers in saliva (8).

Due to increase in the number of diabetes patients globally, a precise and sensitive detection of glucose has drawn the attention of researchers towards the simple and cheap fabrication of glucose sensors (9). The nickel oxide (NiO) nanomaterial with homogenous size and well defined dispersion is highly demanded for the various applications such as designing ceramic, magnetic, electrochromic and heterogeneous catalytic materials (10). The aim of this study is to test if the Nickel Oxide (Nio)/Direct Blue 71 (DB71) Nanoflowers (Nfs) On Glassy Carbon Electrode (GCE) can be used in detection of dextrose in a artificial saliva sample.

2. Materials and Methods:

1. Preparation of NiO

0.1 M (2.5 g) Nickel acetate tetrahydrate ($C_4H_{14}NiO_8$) and 0.2 M (0.8 M) Sodium hydroxide (NaOH) was dissolved in 100 ml of double distilled water (DD H_2O). The solution was stirred well for 30 mins on a magnetic stirrer. The pH was 10, it is a base solution due to the presence of NaOH, which made the solution to precipitate and the formation of Nickel hydroxide (NiOH). To reduce the pH level to neutral, the solution was centrifuged multiple times. Then the sample solution is poured in a petri dish and dried for 24 h at 60 °C. After the sample has dried, it was mortared well until a powdered form was obtained. To convert the amorphous type powder to a crystalline type, the sample powder was calcined for 2 h at 450 °C. After calcination, the sample was again ground well and stored in a 2 ml vial for further characterization and experiment.

2. Preparation of NiO/DB71 composite

0.1 mM (1 mg) of Direct blue 71 (DB71) was dissolved with 10 ml of DD H_2O. Then the dye solution was sonicated in a bath sonicator for 5 mins. 2 mg of NiO was taken and dissolved in a 2 ml of DD H_2O and sonicated for 5 mins. Later, 1 ml of DB71 and 1 ml of NiO soln. was mixed in a 2 ml vial and sonicated for 5 mins.

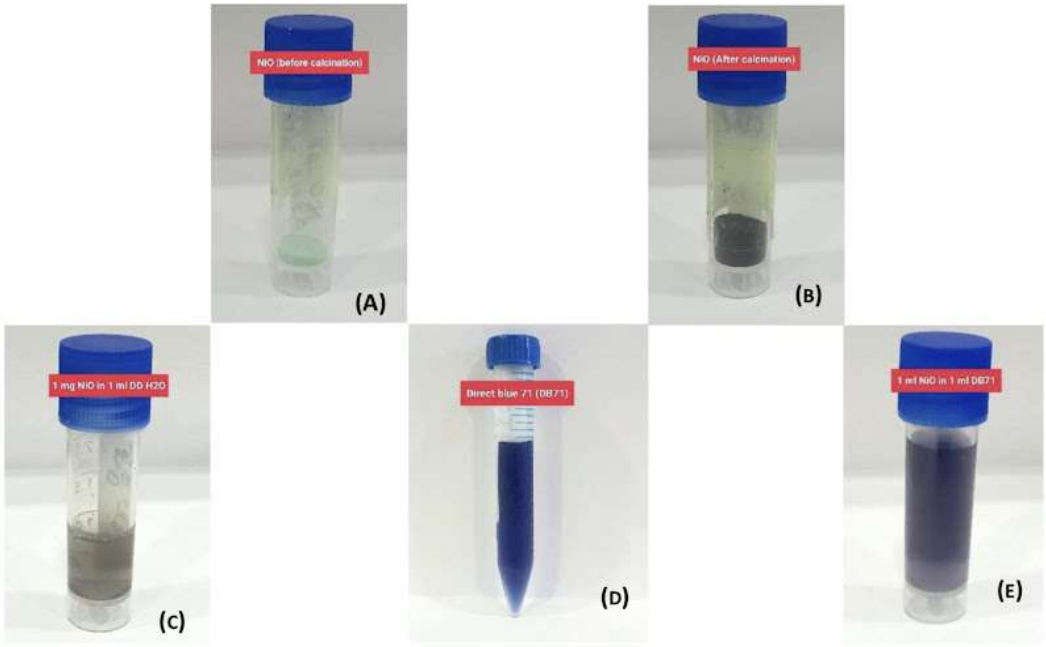

Figure 1: As prepared NiO before calcination, amorphous form (A), Calcined NiO powder, crystalline form (B), 1 mg of calcined NiO dissolved in 1 ml of DD H2O (C), 1 mg direct blue (DB71) in 10 ml of DD H2O (D), 1 mg NiO mixed with 1 ml of DB71 (E).

From figure 1. The prepared mixer soln. was stored in a vial for further experiments and characterizations.

3. Results and discussion:

Material characterization

1. Field emission scanning electron microscope (FESEM)

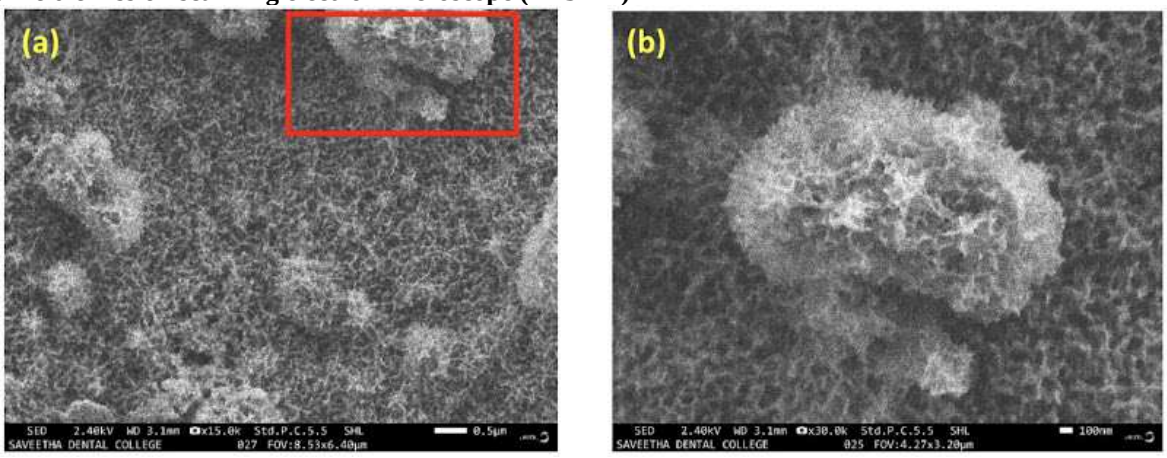

Figure 2: (a) SEM image of NiO/DB71 composite formation, (b) magnified image of NiO/DB71 in a nanoflower form from red mark in fig 2(a).

From figure 2(a). shows a morphology of NiO/DB71 composite formation under 0.5 µm image scale. Figure 2(b) indicates the magnified image of an area marked in fig. 2(a), which is under the magnification image scale of 100 nm and the formation of nanoflowers is confirmed.

Both the figs. 2(a) and (b) have an acceleration voltage of electrons acting on the sample in a FESEM is 2.40 kV.

2. UV-Visible spectroscopy:

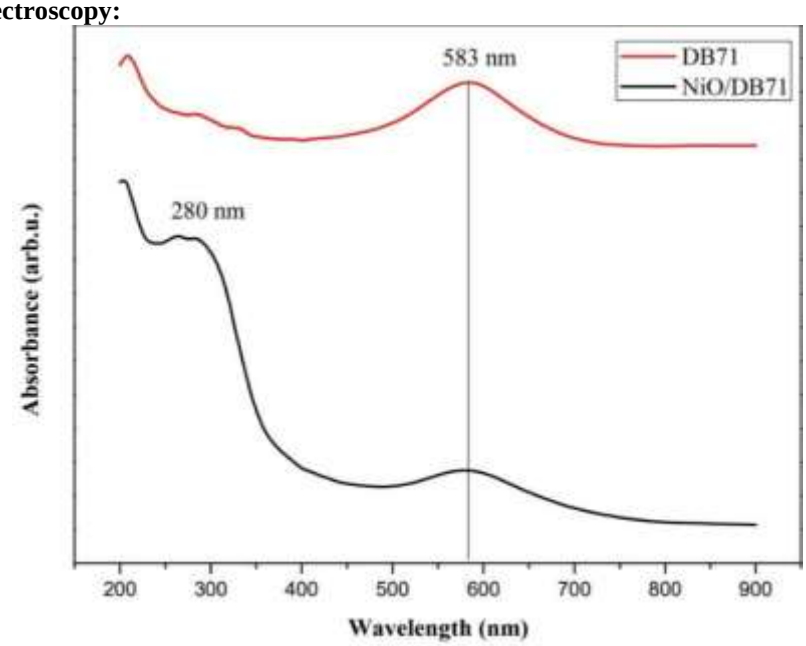

Figure 3: UV Visible spectrum of DB71 (Red), NiO/DB71 composite (Black), the peaks at 280 nm and 583 nm corresponds to the surface plasmon resonance of the composite.

From figure 3. Indicates the UV Visible absorption spectrum of the DB71 and NiO/DB71 composite soln. It is shown that the DB71 dye can sharply absorb light in a visible range of 583 nm, which is yellow color light. And the NiO/DB71 composite have absorbance at 280 nm UV range, which is NiO absorbance, and also in 583 nm visible range. Hence the 583 nm peak in NiO/DB71 confirmed the presence of DB71 dye.

Electrochemical measurements

1. Dextrose detection using NiO/DB71 modified GCE electrode:

a. The modified GCE was run CV in a 0.1 M KOH with 50 µM concentration of dextrose in it and noticed a detection peak around 0.56 V itself.
b. Then further increasing the dextrose concentration to 10 µM, 50 µM, and 100 µM and noticed the increasing current sensitivity up to 7.2 x 10-6 A.
c. To check the impedance of the electrode, both Bare and mod. GCE was tested in an electrochemical impedance spectroscopy (EIS) and noticed that the Mod. GCE showed high impedance, compared to bare GCE.
d. At last, The GCE was polished very well, then the unmodified bare GCE was taken in a 50 µM concentration of dextrose in 0.1 M KOH and noticed that there was no current peak and though it confirmed that the NiO/DB71 material is perfectly suitable for the dextrose detection.

2. Cyclic Voltammogram (CV) of Bare GCE vs Modified GCE comparison for dextrose detection.

a. The well-polished Bare GCE was run in an N2 purged 0.1 M KOH at a potential range of 0.2 V to 0.6 V for 10 cycles first to stabilize the electrode, and after stabilization, one blank run was taken.
b. Then 50 µM of dextrose was added to the 0.1 M KOH electrolyte with an N2 purging, then ran at the same parameter and noticed that the Bare GCE doesn't respond to the dextrose (From figure 3(a)).
c. Further, the GCE was modified with a NiO/DB71, then the same above procedure was followed and noticed a vast difference for a blank and dextrose detection peak, which confirmed the effective electrocatalytic and dextrose detection property of NiO/DB71 combination, (From figure 3(b) and (c))

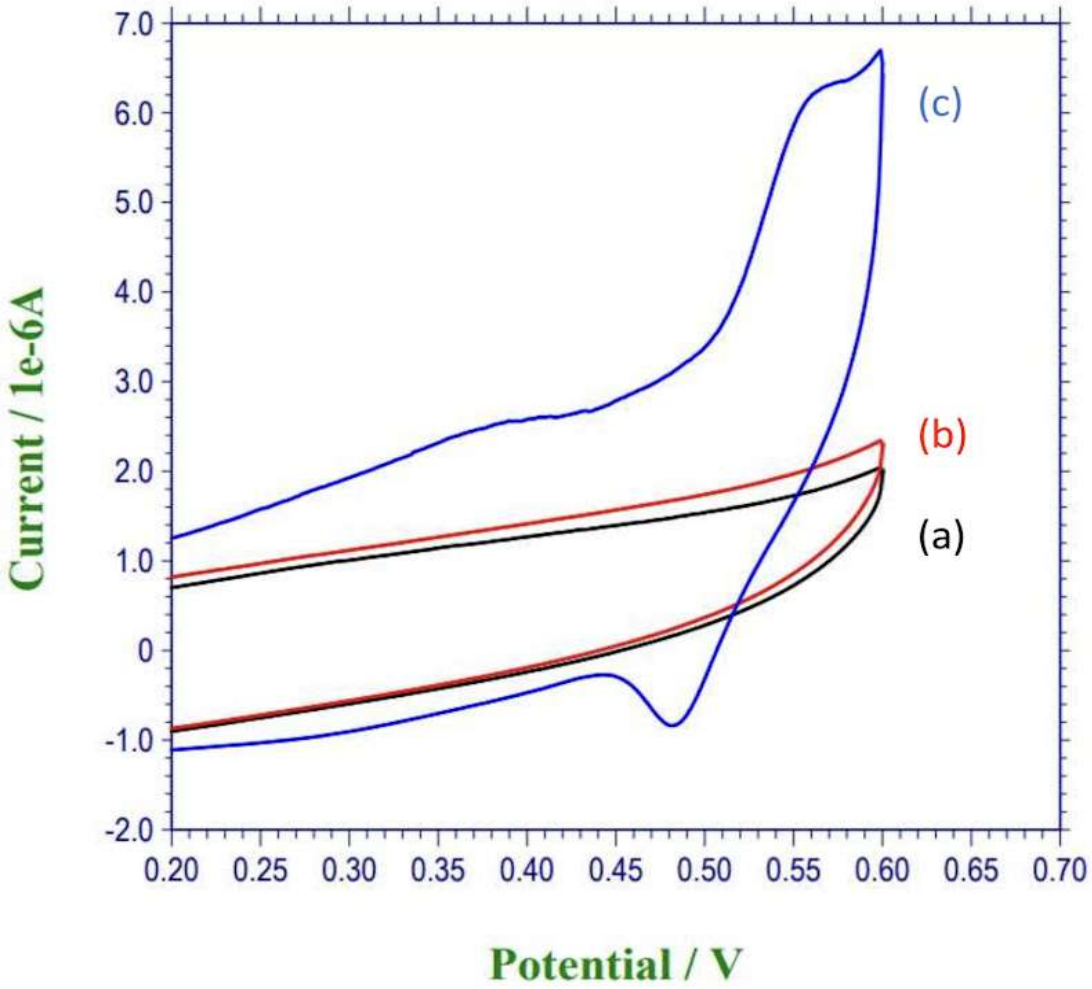

Figure 4: Cyclic voltammogram of Bare GCE in 0.1 M KOH (a), Bare GCE in 50 μm dextrose (b) and NiO/DB71/GCE in 50 μm concentration of dextrose in 0.1 M KOH electrolyte (c) at a scan rate of 50 mV/s.

3. Different concentrations of dextrose were detected by NiO/DB71-modified GCE.

a. The NiO/DB71 modified GCE was run in various concentrations of dextrose (i.e., 10 μM, 50 μM and 100 μM) in 0.1 M KOH.

b. Hence the CV graph of the dextrose detection using the NiO/DB71 and also the only Bare GCE in dextrose contained by 0.1 M KOH is shown in figure 4, in which the response of the modified electrode gave a current peak up to approx. 7.2 x 10-6 A at 0.57 V itself for 100 μM, 6.2 x 10-6 A at 0.56 V for 50 μM and 5.4 x 10-6 A at 0.55 V for 10 μM concentration of dextrose in 0.1 M KOH.

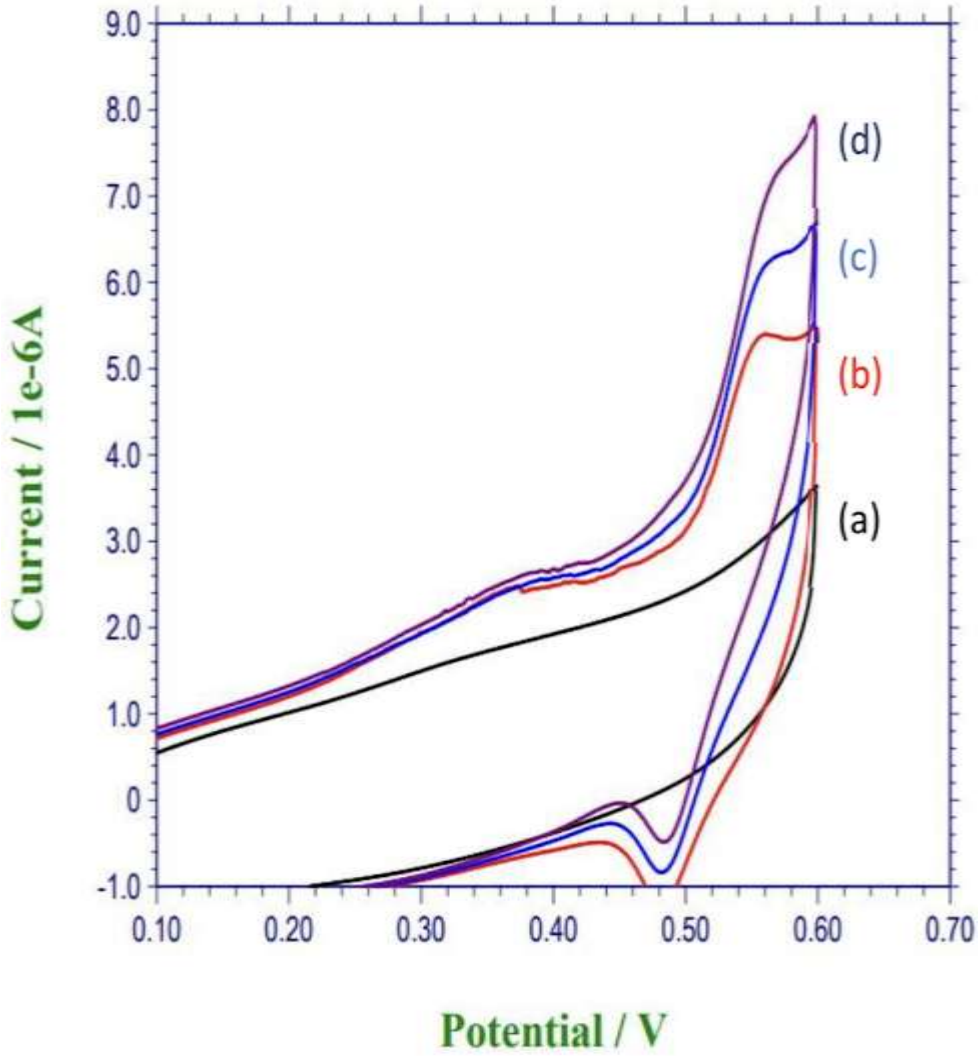

Figure 5 : CVs of NiO/DB71 modified electrode detecting dextrose in different concentration (0 µM (a), 10 µM (b), 50 µM (c) and 100 µM (d)) at 50 mV/s scan rates in 0.1 M PBS electrolyte.

3. The different scan rates of 50 µM concentration of dextrose

a. Added 500 µl of 1 mM dextrose in 0.1 M KOH and purged in N2 gas to remove the excess oxides, therefore 10 ml 0.1 M KOH electrolyte will contain 50 µM concentration of dextrose.

b. CV was taken for different scan rates from 20 mV/s and increased 10 times up to 200 mV/s to study the stability of the material.

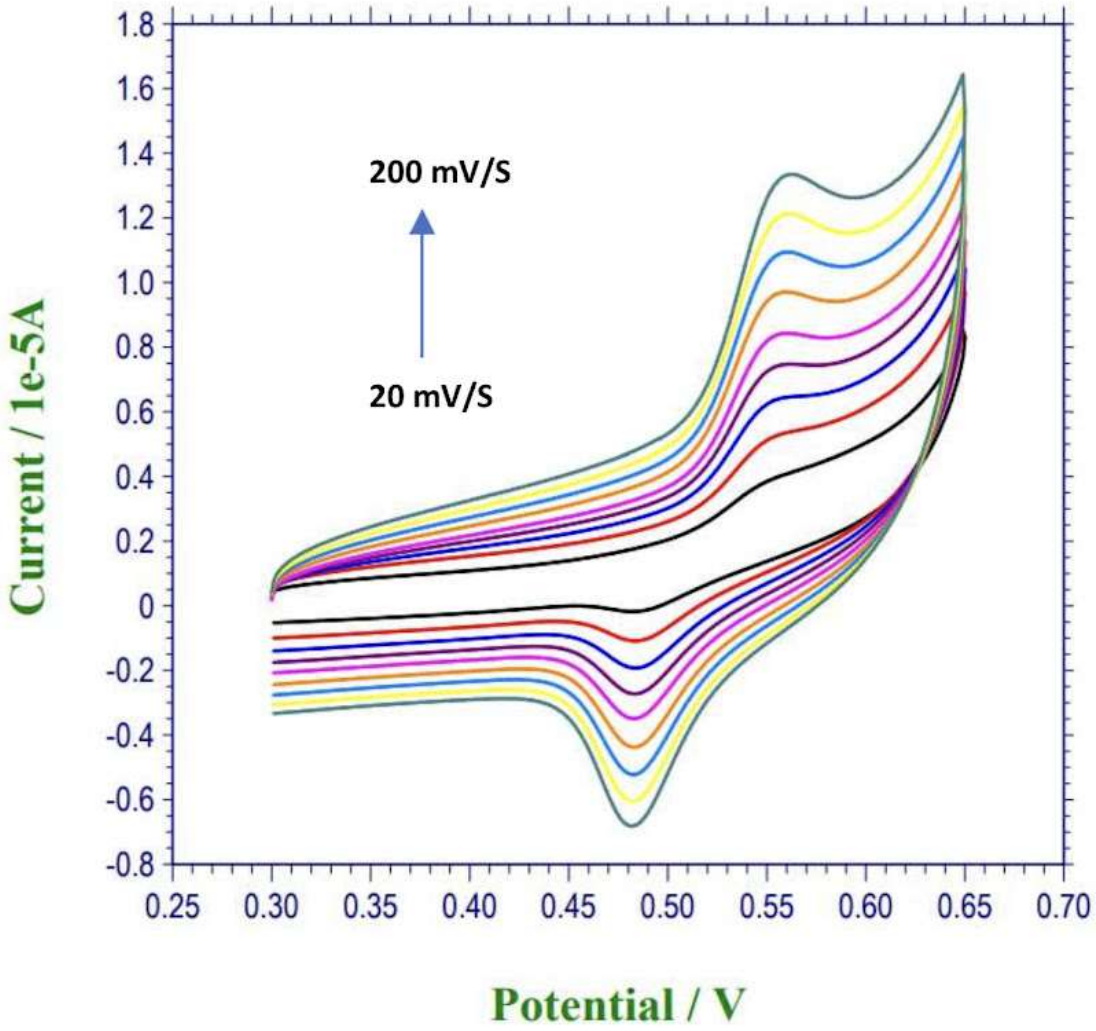

Figure 6 : The different scan rates of dextrose detection from 20 mV/s to 200 mV/s (down to up) for 50 μM concentration of dextrose in 0.1 M KOH.

4. Electrochemical impedance spectroscopy (EIS)

a) From Figure 5. Electrochemical Impedance Spectroscopy (EIS) were shown by comparing bare GCE and NiO/DB71 modified electrode, in which from 0 ohm to 300 ohm there is a solution resistance, whereas the hump indicates the electrodes resistance, for that the resistance of bare GCE is very much lesser than the NiO/DB71 modified electrode, it is because of the resistance of dye molecules presence with DB71 on the modified electrode.

b) The inset plot in figure 6. Indicates the CV comparison of bare GCE vs NiO/DB71 modified electrode in 0.1 M KCl containing 5 mM [Fe (CN)6] 3− / 4− for the confirmation of decrease in current sensitivity and so increase in the resistance of the NiO/DB71 modified electrode.

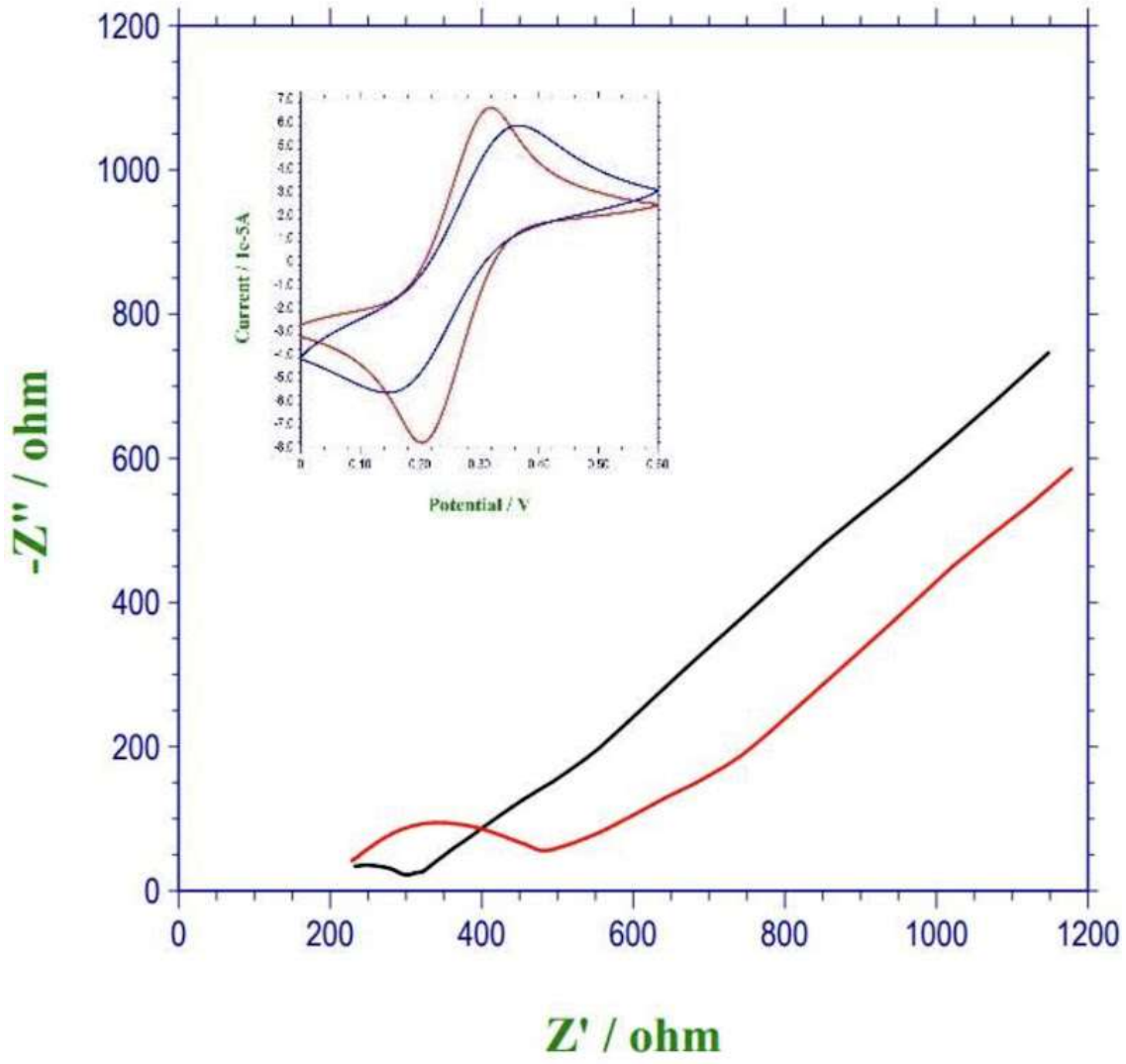

Figure 6: EIS of bare GCE (Black) and NiO/DB71-modified GCE (Red) obtained in 0.1 M PBS. Inset. CVs of bare GCE (Red) and NiO/DB71-modified GCE (Blue) obtained in 0.1 M KCl containing 5 mM [Fe (CN)6] 3− / 4− at a scan rate of 50 mV/s.

5. Conclusion :

Glucose detection is done using a composite of direct blue 71 and nickel oxide . This same procedure is applicable and we can use it as a dextrose detecting biosensor biological samples. The fabricated sensor is also associated with good reproducibility and high stability indicating its high potentiality for the development of enzyme free sensors.

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Conflicts of interest:
The authors declare that there are no conflicts of interest in the present study.

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