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Short Communcation

Molecularly imprinted electrochemical based on NiO nanostructured modified glassy carbon electrode for the electrochemical determination of penicillin in urine of pregnant women infected with Group B Streptococcal

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Molecularly imprinted polymers and a modified glassy carbon electrode (MIP/NiO NPs/GCE) have been shown to be electrochemically sensitive and selective for the measurement of penicillins (PEN) in urine samples from pregnant women using CV and DPV analyses. The NiO NPs were electrodeposited onto the GCE surface to modify it, and a PEN-imprinted polymer was then added to the NiO NPs/GCE. SEM micrographs, XRD patterns, and morphological and structural investigations showed that MIP successfully covered the NiO NPs. According to electrochemical study findings from CV and DPV analyses, NiO NPs modified with MIP shell layers have excellent sensitivity and selectivity for electroanalysis of MIP/NiO NPs matrix nanocomposite. This enhances electrocatalytic activity. The linear concentration range of MIP/NiO NPs/GCE was 0 to 190 µM, with a sensitivity of 0.16237µA/µM and a detection limit of 9 nM. For the purpose of determining the level of PEN in urine samples from pregnant women who were taking PEN medication, the application and validity of MIP/NiO NPs/GCE were investigated. The electrochemical and Penicillin ELISA Kit measurements and the acquired analytical results utilizing the standard addition method were shown to be in good agreement, indicating acceptable levels of recovery (more than 97.00%) and RSD (less than 4.41%). These findings demonstrate the viability of using MIP/NiO NPs/GCE for PEN level assessment in clinical samples.

Keywords: Molecularly imprinted polymers; Electrodeposition; NiO nanoparticles; Penicillin; Pregnant women urine samples; Differential pulse voltammetry

1. INTRODUCTION

Penicillins (PEN, $C_{16}H_{18}N_2O_4S$) are a class of antibiotics that were initially created by the Penicillium fungi and are used to treat bacteria. The PEN differs in the amino and carboxylic side

chains and features a fused ß-lactam thiazolidine ring structure known as 6-aminopenicillanic acid [1]. Based on the mode of action, the carbon atom in the C=O [2] ring of the -lactam ring is obviously essential for its biological activity. Bursting bacteria's cell walls is how PEN functions. The PEN class of drugs operates by subtly rupturing bacterial cell walls [3, 4]. Peptidoglycans, which are crucial for the structural integrity of bacterial cells, and PEN interact to form an irreversible covalent connection [5, 6]. The protein is rendered permanently inactive as a result of the acyl bond formation, which precludes it from performing its roles in cell wall synthesis [7, 8]. The bacterial cell is fatally killed as a result of this deactivation [9, 10].

PEN is used to treat a variety of bacterial illnesses, including skin infections, syphilis, meningitis, ear infections, throat infections, and meningitis as well as to prevent rheumatic fever [11, 12]. Some antibiotics are recognized to be teratogenic and shouldn't be used to treat an infection in a pregnant woman [13, 14]. These include tetracycline, kanamycin, and streptomycin, which may both result in hearing loss (which can lead to weakening, hypoplasia, and discoloration of long bones and teeth) [15]. Women who have contracted group B streptococcal infection have been treated with ß-lactam antibiotics, which include PEN and ampicillin. Patients with soft tissue and bone infections may occasionally require further care, such as surgery [16, 17]. The type of infection brought on by Group B Streptococcal bacteria will determine the course of treatment. PEN can be used in the typical doses throughout pregnancy and breastfeeding [18, 19]. Up to one in three pregnant women have group B streptococci, for which PEN is the preferred antibiotic throughout labor and delivery to avoid major problems for both mothers and babies [20-22]. Antibiotics cannot be administered by doctors prior to the onset of labor due to the bacteria's fast growth. By IV, doctors administer the antibiotic (through the vein). When individuals experience a fever or persistently torn membranes during birth, PEN is also administered [23, 24].

According to the National Birth Disorders Prevention Study, the most frequently administered antibiotic during pregnancy, PEN, as well as other antibiotics, have not been linked to an elevated risk of about 30 different birth defects [25, 26]. However, hypersensitivity events, such as skin rash, hives, edema, and anaphylaxis, or anaphylactic shock, are the main side effects of PEN. Rarely do the more severe reactions occur [27, 28]. Therefore, the clinical applications of the diagnostic sensitivity and determination level of PEN in pharmaceutical samples and biological fluids are important. Many researchers have been interested in developing methods to determine the PEN level in pharmaceutical samples and biological fluids using ELISA [29], high-performance liquid chromatography [30], colorimetric biosensors [31, 32], fluorescent bocillins [33], radioimmunoassay [34], surface-enhanced Raman scattering [35-37], and electrochemical sensors [38-46]. Electrochemical sensors based on molecularly imprinted polymers (MIP) have been demonstrated to be among the most accurate and reliable methods for determining the composition of biological samples that contain a variety of interfering substances, including proteins, enzymes, inorganic and organic compounds [47]. The current research has concentrated on the creation of a nanocomposite of MIP/NiO NPs on the GCE surface.

2. EXPERIMENT

2.1. Preparation of MIP/NiO NPs/GCE

Prior to the modification of the GCE surface, the GCE (3 mm in diameter) surface was successively polished with 0.3 μm alumina powder (99.99%, Sigma-Aldrich) on the polishing pad and then ultrasonically washed with ethanol for 10 minutes, and followed by rinsing with deionized water. An electrodeposition technique was used for modification of the GCE surface by NiO nanoparticles using an electrochemical workstation potentiostat (CHI660D, Shanghai Chenhua Instrument Ltd., Shanghai, China) in a three-electrode electrochemical setup [48]. The electrochemical setup was contained GCE, an Ag/AgCl (3 M KCl) and platinum plate as working, reference and counter electrode, respectively. Electrodeposition was performed in electrolyte containing equel volume ration of 0.1 M H_2SO_4 (97%) and 0.2 M $NiSO_4$ (99%) by applying a constant potential electrolysis at −1.25 V for 2 minutes. Then, the electrode surface was washed with deionized water and followed by air drying for one hour.

For modification of the NiO NPs/GCE with PEN-imprinted polymer [49], 0.05 g PEN and 0.07 g methacrylic acid (99%, Sigma-Aldrich) were ultrasonically added in 4 mL of dimethyl sulfoxide (≥99.9%, Sigma-Aldrich). After 4 hours of ultrasonication, 1.03 g of ethylene glycol maleic rosinate acrylate (99 %, Sigma-Aldrich) and 0.01 g of 2, 2 -Azobisisbutyronitrile (99 %, Sigma-Aldrich) were ultrasonically added into the above mixture to achieve PEN -imprinted polymer solutions. Then, the 100 μL PEN-imprinted polymer solution was dropped on the NiO NPs/GCE surface followed by thermal polymerization in vacuum oven at 70 °C for 5 hours. After that, to remove the template molecules, the imprinted electrode was washed for 6 minutes with a mixture of acetic acid (99 %, Sigma-Aldrich) and methanol (≥99.9%, Merck, Germany) in a volume ratio of 3:7. Afterwards, the electrode was washed with deionized water three times before being used.

2.2. Instruments

The characterization of manufactured NiO NPs and MIP/NiO NPs composites used X-ray Diffraction (XRD, 7000, Shimadzu-Scientific-instruments, Japan) and scanning electron microscopy (SEM, JSM-7800F, JEOL, Japan). The electrochemical workstation potentiostat (CHI660D, Shanghai Chenhua Instrument Ltd., Shanghai, China) in a three-electrode electrochemical setup was utilized for the electrochemical determination of PEN by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) approaches. All electrochemical experiments were conducted in a 0.1M phosphate buffer solution (PBS) electrolyte (pH 7.4), which was made up of an equal volume ratio of 0.1M NaH_2PO_4 (99%, Sigma-Aldrich) and 0.1M Na_2HPO_4 (99%, Sigma-Aldrich).

2.3. Analysis of actual sample

Electrochemical analysis was used to evaluate the concentration of PEN in urine samples from pregnant women who were using PEN medication. The supplied urine samples were centrifuged at

1500 rpm for 5 minutes and filtered using filter paper before electrochemical tests. Finally, 0.1M PBS was made using the filtered urine samples (pH 7.0). The level of PEN in patient urine samples was also determined using the Penicillin ELISA Kit. Using the usual addition approach, the recovery and relative standard deviation (RSD) values were obtained.

3. RESULTS AND DISCUSSION

3.1. Morphological and structural analyses

The morphological structures of NiO NPs and MIP/NiO NPs modified GCE, respectively, are shown in SEM micrographs in Figures 1a and 1b. NiO NPs are electrodepositioned on the GCE electrode surface in spherical shapes with an average size of 50 nm, according to SEM micrographs of NiO NPs/GCE. SEM micrographs show that the electrode surface had a modest alteration in the appearance of NiO NPs when covered with MIP after the MIP polymer had been immobilized on the surface of NiO NPs/GCE. GCE modified MIP/NiO NPs had an average size of 60 nm. It is evident that the MIP/NiO NPs/GCE surface has micro-pores, which facilitate the electrochemical reaction by providing a route for the insertion and extraction of electrolyte ions and improve the efficiency of liquid-solid interfacial area.

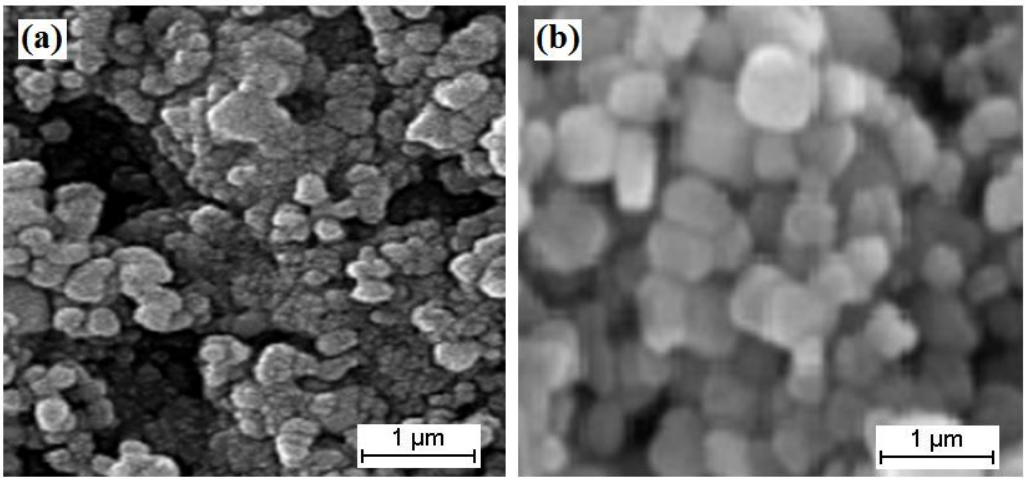

Figure 1. SEM micrographs of the morphological structures of (a) NiO NPs and (b) MIP/NiO NPs modified GCE.

Figure 2 displays the findings from X-ray diffraction measurements of the phase purity and crystallinity of powders of NiO NPs and MIP/NiO NPs. The face-centered cubic phase structure of NiO is shown by distinctive strong diffraction peaks in the XRD profile of NiO NPs at 37.37°, 43.41°, and 62.90°, respectively (JCPDS card No. 74-1049) [50-52]. The diffraction peaks of (111), (200), and (220) are shown in the XRD profile of MIP/NiO NPs with reduced density, proving the polymer's amorphous nature [53-56] and successfully covering the NiO NPs.

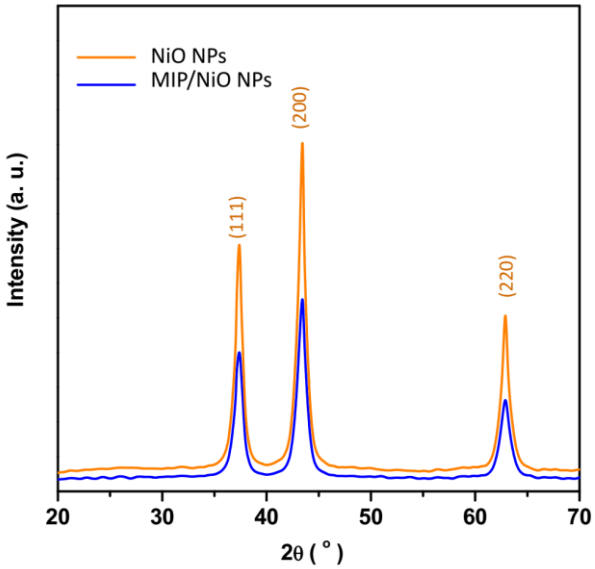

Figure 2. Results of structural characterization of powders of NiO NPs and MIP/NiO NPs using X-ray diffraction technique.

3.2. Electrochemical studies

Figure 3 shows the CV curves of unmodified GCE, NiO NPs/GCE, and MIP/NiO NPs/GCE at potentials between 0.40 V and 0.80 V at a scanning rate of 20 mV/s in 0.1 M PBS (pH 7.0) in both the absence and presence of PEN solution. It has been noticed that all of the electrodes do not exhibit a distinctive peak in the CV curves when the PEN solution is absent. The anodic peaks on the CV curves of GCE, NiO NPs/GCE, and MIP/NiO NPs/GCE in the presence of a 70 M PEN solution are attributed to the oxidation of the sulfide moiety of the-lactam ring and the formation of a sulfoxide derivative by the involvement of water, two protons, and the transfer of two electrons, as shown in Figure 4 [57-59]. As can be seen, the electrocatalytic current of MIP/NiO NPs/GCE is larger than that of NiO NPs/GCE and bare GCE, and the peak current of MIP/NiO NPs/GCE occurs at the lowest potential. It has to do with the molecularly imprinted polymer matrix and the synergistic electrocatalytic effect of NiO nanoparticles. NiO NPs, for example, with a large surface area and high electrical conductivity can increase electrocatalytic current and provide electrons with direct conducting pathways, but more importantly, they can provide enough loading surface to decorate MIPs and improve sensitivity [60-64]. The MIP/NiO NPs matrix nanocomposite offers surface-initiated activators that renew via electron transfer for atom transfer radical polymerization over the NiO NPs and create MIP shell layers tethered to NiO NPs cores [65]. Because cavities with complementary binding sites are formed in the imprinted polymer matrix, the electrocatalytic currents are increased and can more easily bind the target analytes [66-68]. Therefore, the electrocatalytic activity and/or outstanding sensitivity for electroanalysis of MIP/NiO NPs matrix nanocomposite are promoted by modification of NiO NPs with MIP shell layers. Thus, employing MIP/NiO NPs/GCE, the following electrochemical tests were conducted.

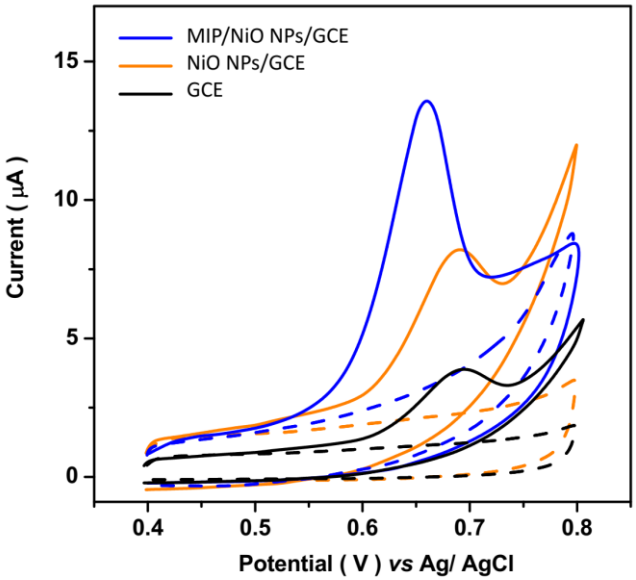

Figure 3. The CV curves of unmodifed GCE, NiO NPs/GCE and MIP/NiO NPs/GCE at the potential range from 0.40 V to 0.80 V at scanning rate of 20 mV/s in 0.1 M PBS (pH 7.0) in absence (dashed line) and presence (solid line) of 70 μM PEN solution.

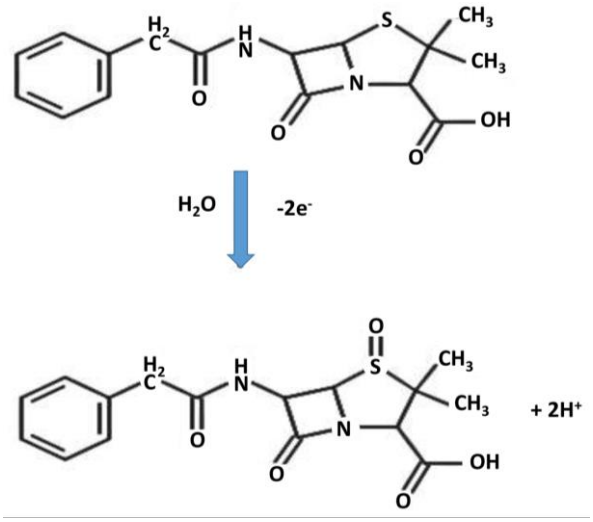

Figure 4. The suggested mechanism of electro-oxidation of PEN [57].

In order to characterize the mass transport in the diffusion layer of MIP/NiO NPs/GCE, the effect of scan rate on the peak current was studied. Figure 5 exhibits the CV curves of MIP/NiO NPs/GCE at the potential range from 0.40 V to 0.80 V in 0.1 M PBS (pH 7.0) containing 70 μM PEN solution at a scanning rate of 5 to 200mV/s. As seen, the anodic peak current of 70 μM PEN was increased with an increase in scan rate. From the inset of Figure 5, it can also be seen that peak current is linearly proportional (R^2= 0.9998) to the square root of the scan rate within the range of 5 to 200 mV/s, implying that the mass transport is controlled by diffusion thus proving that the rate-limiting

adsorption and/or specific interactions on the MIP/NiO NPs/GCE surface are negligible [69]. The slight shift of peak potential towards more positive potential was observed as the scan rate increased.

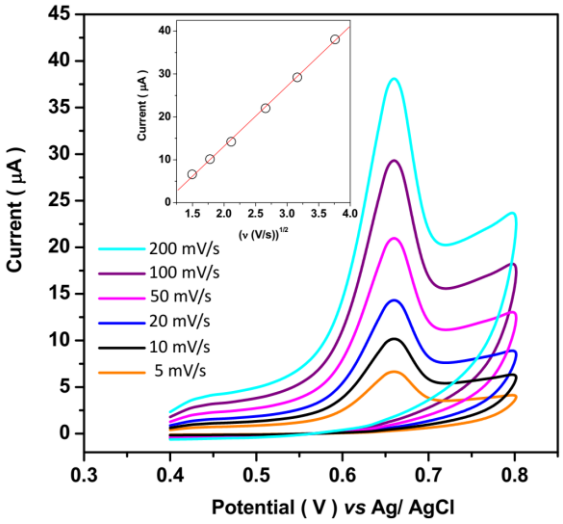

Figure 5. CV curves of MIP/NiO NPs/GCE at the potential range from 0.40 V to 0.80 V in 0.1 M PBS (pH 7.0) containing 70 µM PEN solution at scanning rate fron 5 to 200 mV/s.

Figure 6 shows the DPV responses of MIP/NiO NPs/GCE to injections of 10 µM PEN solutions at potentials ranging from 0.48 V to 0.80 with a scanning rate of 20 mV/s in an electrochemical cell containing 0.1 M PBS (pH 7.0). The DPV peak current intensity at 0.66 V is shown to rise with each injection of 10 M PEN solution. The calibration plot shown in Figure 5 shows that the peak current intensities of DPV responses rise linearly over the concentration range of 0 to 190 µM, and a sensitivity of 0.16237 µA/µM for MIP/NiO NPs/GCE toward PEN is obtained. Additionally, a 9 nM estimate for the detection limit can be made. The outcomes are contrasted with recently released PEN data in Table 1. As can be seen, MIP/NiO NPs/GCE as a wide linear range electrochemical sensor in voltammetric studies is considered a novelty in electrochemical platforms. Additionally, the MIP/NiO NPs/GCE in the presence study exhibits an acceptable detection limit value because the MIP/NiO core-shell structure as a magnetic molecularly imprinted polymer provides good electrical properties and straightforward chemical functionality for the development of compatible biomimetic receptors on the electrode surface, as well as the existence of high-quality specific electroactive sites for recognition/capture of PEN molecules [44, 70]. Moreover, the MIP/NiO core-shell structure delivers a large surface area and high stability as well as enhancement of sensor stability and signal amplification [44, 71].

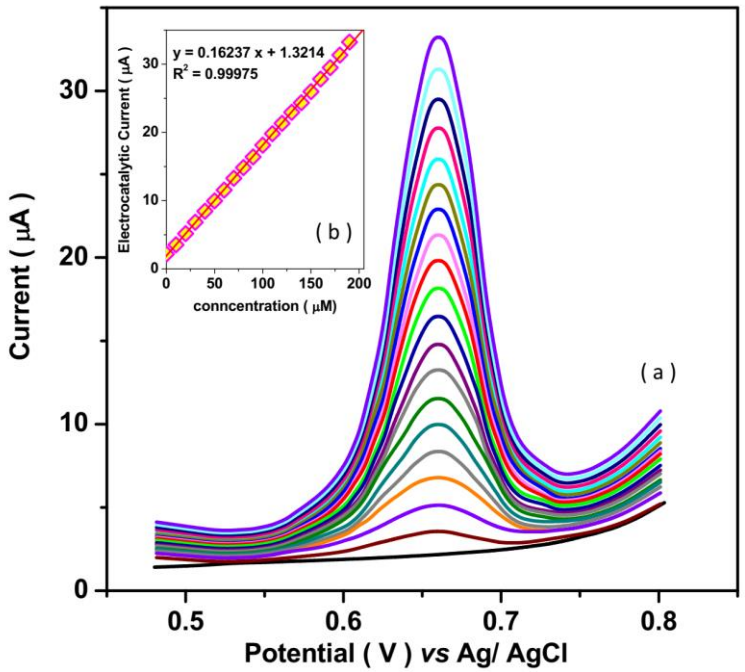

Figure 6. (a)The DPV responses of MIP/NiO NPs/GCE at the potential range from 0.48 V to 0.80 V with a scanning rate of 20 mV/s to each injection of 10 µM PEN solutions in electrochemical cell containing 0.1 M PBS (pH 7.0); (b) calibration plot.

Table 1. Comparison between obtained sensing parameters of MIP/NiO NPs/GCE and recent reported PEN electrochemical sensors.

Electrodes	**Technique**	**Detection limit (nM)**	**Linear range (µM)**	**Ref.**
MIP/NiO NPs/GCE	DPV	9	0 to 190	This work
Boron doped diamond electrode	DPV	250	0.5 to 40	[39]
Ni NPs/ screen-printed carbon electrodes	DPV	0.31	0.01 to 0.5	[43]
Boron doped diamond electrode	SWV	320	0.4 to 100	[38]
TiO_2 NPs/ Carbon Ionic Liquid Electrode	SWV	2.09	0.003 to 1	[40]
Thin film antimony-antimony oxide enzyme electrode	Potentiometry	---	300 to 7000	[42]
Penicillinase enzyme/ZnO nanorods/gold coated glass substrate	Potentiometry	---	10^2 to 10^5	[45]
methylene blue/Horseradish peroxidase-labeled penicillin polyclonal antibody/GCE	CV	1.82	0.0052 to 0.0416	[72]

SWV: Square wave voltammetry

The specificity of the MIP/NiO NPs/GCE system was examined for the purpose of determining PEN in the presence of various chemicals found in biological liquids. Table 2 shows the outcomes of electrocatalytic signal of DPV measurement at a potential range of 0.48 V to 0.80 V with a scanning rate of 20 mV/s in 0.1 M PBS (pH 7.0) to sequential injections of 10 µM PEN solution and 60 µM of

substances. Due to interferences arising from insufficient specificity of the electrode surface and type of electrolyte, as well as when these chemicals reduce and/or oxidize at potentials near to the PEN, interferences can occur [73-76]. It has been found that adding MIP/NiO NPs/GCE to the PEN solution produces a notable electrocatalytic signal. However, adding interfering chemicals to the electrolyte solution has no discernible effect on the electrocatalytic signal of the PEN. Because monomers and the integration of a template molecule are employed in the molecular imprinting modification approach, it can be determined that the MIP/NiO NPs/GCE can be viewed as a particular PEN sensor. The polymer matrix develops voids after the template removal procedure. High selectivity is present for the template molecule in these cavities [24, 77, 78]. Better accessibility of the particular receptor-recognition contacts for the analyte and less resistance to mass transfer are provided by the synergistic effect of the recognition sites of magnetic molecularly imprinted polymer for the analyte and NiO NPs [77, 79]. As a result, an improved electrochemical sensing system with high PEN sensitivity and selectivity is formed by the nanostructured MIP/NiO core-shell structure [80, 81].

Table 2. The results of electrocatalytic signal of DPV measurement of MIP/NiO NPs/GCE at the potential range from 0.48 V to 0.88 V with a scanning rate of 20 mV/s in 0.1 M PBS (pH 7.0) to successive injections of 10 µM PEN solution and 60 µM of substances.

Substance	**Added (µM)**	**Electrocatalytic signal (µA)**	**RSD**
PEN	10	1.6240	±0.0130
Glucose	60	0.0550	±0.0012
Roxithromycin	60	0.0611	±0.0015
Ascorbic acid	60	0.0512	±0.0011
Clindamycin	60	0.0421	±0.0016
Ampicillin	60	0.0284	±0.0015
Uric acid	60	0.0262	±0.0017
Cloxacillin	60	0.0352	±0.0011
K^+	60	0.0447	±0.0013
Mg^{2+}	60	0.0695	±0.0011
NO_3^-	60	0.0314	±0.0010
Zn^{2+}	60	0.0712	±0.0021
Ca^{2+}	60	0.0444	±0.0044
PO_4^{3-}	60	0.0411	±0.0032
SO_4^{2-}	60	0.0643	±0.0041
Fe^{3+}	60	0.0431	±0.0017

For the purpose of determining the level of PEN in urine samples from pregnant women who were taking PEN medication, the application and validity of MIP/NiO NPs/GCE were investigated. Results from a DPV measurement performed on urine samples prepared in 0.1 M PBS (pH 7.0) at potentials between 0.48 V and 0.8 V with a 20 mV/s scanning rate. The analytical results utilizing the standard addition method are shown in Table 3. They show good agreement between the results from measurements made using electrochemical and Penicillin ELISA Kits and indicate appropriate

recovery (more than 97.00 %) and RSD values (less than 4.41%). These findings demonstrate the viability of using MIP/NiO NPs/GCE for PEN level assessment in clinical samples.

Table 3. The obtained analytical finings using electrochemical and Penicillin ELISA Kit measurements for determination PEN in prepared real samples of pregnant women urine samples.

	Electrochemical measurement			**Penicillin ELISA Kit**		
spiked (µM)	**detected (µM)**	**Recovery (%)**	**RSD (%)**	**detected (µM)**	**Recovery (%)**	**RSD (%)**
0.00	0.05	---	4.41	0.06	---	3.34
1.00	1.02	97.00	4.29	1.04	98.00	4.52
3.00	3.03	99.33	3.74	3.04	99.33	3.68
5.00	5.01	99.20	3.65	5.01	99.00	4.28

4. CONCLUSION

The goal of the current study is to create a nanocomposite of MIP/NiO NPs on the surface of GCE that can be employed as a sensitive and specific PEN electrochemical sensor to measure the level of PEN in urine samples from pregnant women. To modify it, the NiO NPs were electrodeposited onto the GCE surface. The NiO NPs/GCE was subsequently modified using a PEN-imprinted polymer. The NiO NPs were successfully covered with MIP, as shown by SEM micrographs and XRD pattern studies. The electrochemical tests' findings showed that adding MIP shell layers to NiO NPs enhanced their electrocatalytic activity and provided excellent sensitivity and selectivity for electroanalysis of the MIP/NiO NPs matrix nanocomposite. The linear concentration range of MIP/NiO NPs/GCE was 0 to 190 µM, with a sensitivity of 0.16237µA/µM and a detection limit of 9 nM. For the purpose of determining the level of PEN in urine samples from pregnant women who were taking PEN medication, the application and validity of MIP/NiO NPs/GCE were investigated. The results of the electrochemical and Penicillin ELISA Kit measurements were shown to be in good agreement with the acquired analytical results using the standard addition method, indicating acceptable levels of recovery and RSD. These findings demonstrate the viability of using MIP/NiO NPs/GCE for PEN level assessment in clinical samples.

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