Int. J. Electrochem. Sci., 9 **(2014)** 6201 - 6212

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

A Penicillin Biosensor by Using Silver Nanoparticles

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Received: 19 July 2014 / *Accepted:* 21 August 2014 / *Published:* 25 August 2014

Based enzymatic biosensor for selective detect of penicillin by using silver nanoparticles designed. Convergence of bioelectrochemistry and nanotechnology improved duty of novel biosensors. Here silver nanoparticles used to facilitate electron exchange between biosensor elements and investigated with XRD, TEM and size distribution techniques and the mean size of the silver nanoparticles was around 90 ± 10 nm. Main equation for this research was: Penicillin + H_2O = penicilloate^{-}+ H^{1+}. The electrochemical evaluations applying the potentiometric method were carried out for different penicillin G salt concentrations varying from 100 µM to 100 mM made in a buffer solution at pH 7.4. The tested sensor configuration showed a wide linear dynamic range for the output response *vs*. the logarithmic concentration of penicillin G salt solution. To investigate the reproducibility of the given sensor, we examined the potentiometric reply of all five sensor electrodes in 5, 10, 20 and 30 mM penicillin solutions also the relative standard deviation was detected to be less than 4%.

Keywords: Biosensor, Penicillin, Silver nanoparticles, Electrochemistry

1. INTRODUCTION

Nanoelectrochemistry is a branch of electrochemistry that experiments the electrical and electrochemical characteristics of substances at the nanometer size regime. Nanoelectrochemistry performs definitive responsibility in the fabrication of various sensors, and devices for detecting molecules at very low concentrations [1]. nanotechnological attempts start to overcome in biochemistry throughout analyzes of biological materials. Novel nanotechnologies compose selectivity as well as specificity of natural biochemistry moreover application of minor volumes of tastes and excessively low concentrations of assayed biological substances [2-3]. Silver is much cheaper also less

infrequent than gold, with a sector of 0.1 mm-thick silver foil costing a fifth of the measure one would disburse for a sector of gold foil a quarter of the size. It is harder but still extremely ductile and malleable [4]. Pure silver has the highest electrical and thermal conductivity of all metals and possesses the lowest contact resistance. Silver is of great importance in electrochemistry and is used in reference and counter electrodes, in the manufacture of other electrodes through its use as electrical contact material and as silver paint for printed electrical circuit boards [5]. Silver is stable in air and water, but tarnishes when exposed to ozone, hydrogen sulfide, or air containing sulfur. Macro silver electrodes have been applied to detect hydrogen peroxide, cyanide, sulfide, iodide, and bromide and organic combinations such as enzymes, DNA and hemoglobin. Bulk silver has been demonstrated to display catalytic activity for hydrogen peroxide through various pathways; here we used of silver nanoparticles to facilitate electrochemical events in designed biosensor [6-7]. The development of biosensors was driven by the need for faster and more versatile analytical methods for application in important areas including clinical, biomedical, environmental, industrial and pharmaceutical analysis. The biosensors are an attractive alternative to conventional analytical methods, such as liquid chromatography, used for determination of phenolic compounds in several types of the samples. Some advantages of biosensors, relative to chromatographic techniques, are their hurrying reply, cost-effectiveness, facility of application as well as producing [8]. It is identified that a cautious selection of the biological receptor and operated potential much enhances their selectivity and sensitivity [9]. Enzyme electrodes are one of the best intensively experimented biosensors due to enzymes are highly selective additionally reply rapidly to a particular substrate. The experiment done on enzyme field-effect transistors (EnFET) by Caras and Janata [10] has steered the trend towards potentiometric biosensors based on a semiconductor structure to become very popular [11-12].Many potentiometric devices are based on various forms of FET devices to measure pH changes, selective ion concentrations, and the kinetics of biocatalytic reactions including enzymes [13]. The change of a FET into a sensing device ordinarily includes the supersession of the metal gate electrode by a biochemically sensitive surface (e.g., an analyte-selective membrane, an enzyme layer or an ion conductive solution, etc.), which is brought into touch with the solution to be detected [14].In the ancient, a biosensor based on the penicillinase enzyme immobilization on the external of transducer by homo-functional cross-linking with glutaraldehyde technique, has been described [15].The inference for this might be because the inter enzyme molecules cross linking instead of the attaching of the enzyme molecules to the transducer surface [16]. There is also another method used for the immobilization of penicillinase enzyme which is the hetero-functional cross-linkers [17]. This is a two-step method, in the first step cross linker molecules bind to the pH sensitive transducer and after that enzyme molecules are added to them. The potentiometric technique is easy, quick, and of low cost for the detection of penicillin by applying the ion selective electrodes. Furthermore, potentiometric sensors have been applied to determine individual as well as all measures in diverse β-lactam assaying analytics [18]. In the present analyze, we have beneficially displayed the fabrication of a silver nanoparticles based biosensor with good reproducibility and selectivity for fast monitoring of penicillin with immobilization of penicillinase enzyme by simple physical adsorption method.

2. EXPERIMENTAL SECTION

2.1. Materials

The penicillinase enzyme with given activity 1,500–3,000 U/mg-protein from Bacillus cereus, penicillin G sodium salt, sodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl), potassium chloride (KCl), sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from (Sigma Aldrich). Silver nitrate ($AgNO_3$), Hydrazine hydrate, Citrate of sodium and Sodium Dodecyl Sulphate (SDS) were purchased from Merck. The N-5-azido-2-nitrobenzoyloxysuccinimide (ANB-NOS) cross linking chemical was purchased from (Pierce). All other chemicals were of analytical grade.

2.2. Apparatus

All the electrochemical experiments were carried out using potentiostat PGSTAT 30 model from Autolab (Ecochemie, Netherlands), interfaced with a private computer. All the potentials were described regarding to an Ag/AgCl reference electrode. The morphology of the produced particles was done on a JEOL JEM-1200 EXII transmission electron microscope operating at accelerating voltages of 120 kV. The XRD experiments were carried out on a SIEMENS D500 diffractometer equipped with Cu Kα R radiation (λ = 1.5406 A). The electrochemical cell was a three-electrode system consisting of a glassy carbon working electrode (diameter of 3 mm), a platinum counter electrode, and an Ag/AgCl (3MNaCl) reference electrode. All pH measurements were done on a Jenco digital pH meter. Also, the experiments were carried out inside a Faraday cage at room temperature (ca. 25 °C).

2.3. Preparation of silver nanoparticles

For the composition of silver nanoparticles two stabilizing factors, Sodium Dodecyl Sulphate (SDS) and Citrate of sodium were used. For the synthesis of silver nanoparticles, silver nitrate solution (from 1,0 mMto 6,0 mM) and 8% (w/w) Sodium Dodecyl Sulphate (SDS) were used as a metal salt precursor and a stabilizing agent, respectively. Hydrazine hydrate solution with a concentrate ranging from 2,0mM to 12 mM also Citrate of sodium solution (1,0 mM to 2,0 mM) were applied as a diluting factors. Citrate of sodium was also applied as fixing factor at room temperature. The transparent colour less solution was changed to the definite pale yellow and pale red colour, when citrate of sodium was applied as fixing factor. The existence of colour was demonstrated the creation of silver nanoparticles. The silver nanoparticles were purified by centrifugation. To remove excess silver ions, the silver colloids were washed at least three times with deionized water under nitrogen stream. A dried powder of the Nanosize silver was acquired by freeze-drying. To carry out all improvisation techniques also interaction of the silver nanoparticles with bacteria, the silver nanoparticle powder in the freeze-drying cuvette was resuspended in deionized water; the suspension was homogenized with a Fisher Bio block Scientific ultrasonic cleaning container.

2.4. Immobilization of the Enzyme

sensor electrodes were prepared after the immobilization of enzyme penicillinase on to the surface of grown silver nanoparticles in combination with (ANB-NOS) as a cross linker. The process of immobilization followed two steps; first a 10 mM (ANB-NOS) solution was prepared in phosphate buffer at pH 7.4, then silver nanoparticles electrode was hatched in this solution for one hour, after that sensor electrode was washed with de-ionize water to remove the solid residue particles, then in second step, this electrode was put into the enzyme penicillinase solution for 20 min. The penicillinase enzyme solution was developed in same phosphate buffer at pH 7.4 additionally concentration of enzyme was 5 mg/mL.Then immobilized sensor electrode was kept at 4 °C temperature for about 16 hours. All immobilized sensor electrodes were kept at 4 °C temperature when not in use.

2.5. Preparation of Sensor Working Electrode

The most commonly used carbon-based electrode in the analytical laboratory is glassy carbon (GC). It is made by pyrolyzing a carbon polymer, under carefully controlled conditions, to a high temperature like 2000 ^{o}C. An intertwining ribbon-like substance effects with retention of high conductivity, firmness and inertness. The electrochemistry is influenced extensively by its surface chemistry of carbon-oxygen functionalities and its cleanliness; i.e., lack of adsorbed imperfections. Polishing on smooth ground glass plates enable the circumstance of the polishing substance to be rigidly managed for optimum cleanliness. In addition, the use of deagglomerated alumina of small particle size (0.05 μm) allows for a more scratch free active electrode surface. The use of particles of even smaller size (0.007 μm fumed silica) should improve the facility also behavior. If this does not activate the surface, re-polishing with larger particle size abrasive, such as 0.25: m diamond ensued by 0.05: m alumina or 0.007: m silica. The degree of activation of a GC disk electrode can be evaluated by looking at the diversity in the peak potential for the redox couple of ferri/Ferrocyanide, which is expected to be close to 60 mV. First, use slurry of the 0.05 μm alumina to polish the electrode. Polish with light pressure for nearly 30 seconds. The electrode should next be instantly rinsed with water. GC electrodes can maintain much of their behavior by saving them in a solution of alumina or silica. To act this, add 6 or 7 scoops of both alumina and silica to about 25 ml of deionized water additionally shake the solution with the electrode in it. Basically, the electrode is being continuously polished. When you are ready to use the electrode again, rinse it with a stream of pure deionized water and it is ready to go.

3. RESULTS AND DISCUSSION

3.1. Transmission electron microscopy investigation

The transmission electron microscopy (TEM) is a Special tool that considered in determining the structure and morphology of the nanomaterial and provides the structure of materials with high spatial resolution & magnification. In addition, this microscope can be used to study the crystal

structure, symmetry, orientation and crystalline defects. These features have led the TEM nowadays be a very important tool in many advanced research in physics, chemistry, crystallography, materials science and biology. In this research the morphology of the well aligned and vertically oriented increased silver nanoparticles was analyzed by Transmission electron microscopy (TEM) as demonstrated in Figure 1; for this assays the scales bare was 100 nm.

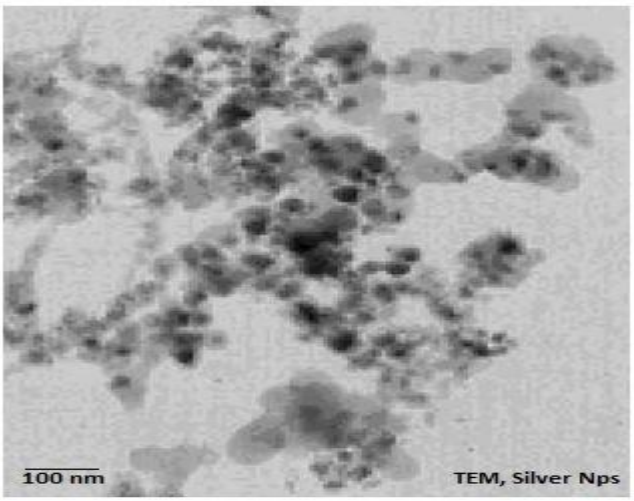

Figure 1. Transmission electron microscopy investigation of silver nanoparticles; scale bare was 100 nm.

3.2. X-ray diffraction (XRD) investigation

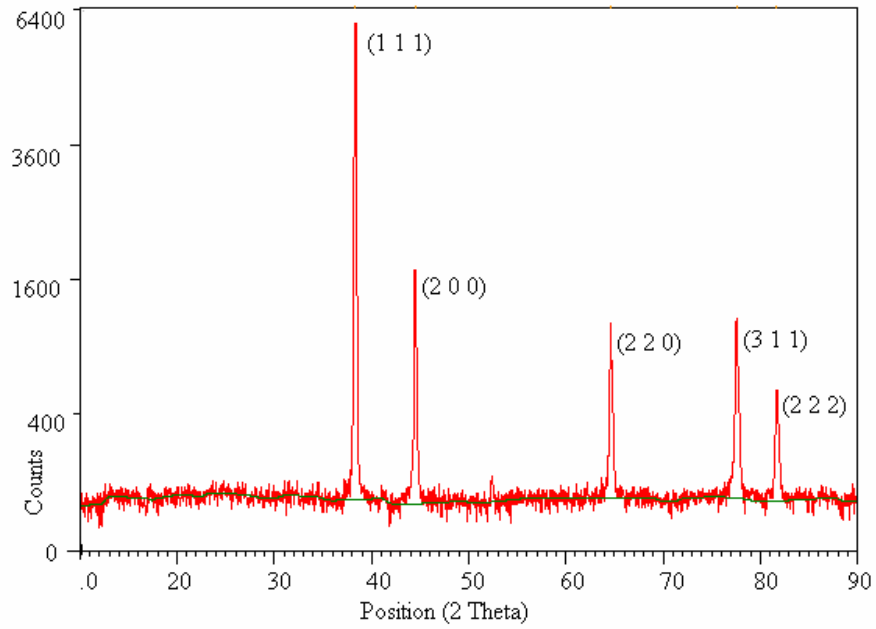

Figure 2. XRD pattern for silver nanoparticles

The powder XRD pattern of the silver nanoparticle is shown in Figure 2. Here, the three peaks can be seen at 2θ= 38.2,44.3 and 64.5o, which are characteristic diffraction peaks of metallic silver. These peaks dispatch to the three d-spacing (111), (200), additionally (220), respectively. Crystallite size (D) is measured from Scherrer's equation $D = K\gamma/(\beta\cos\theta)$, for peak expanding from size results only. According to the equation of Scherrer's equation, the mean diameter of silver nanoparticles arranged was about 90nm.

A particle size analyzer was applied to determine the area of sizes of the silver nanoparticles. Figure 3 demonstrates the size dispersion of one of the arranged silver nanoparticles. The mean size of the silver nanoparticles was around 90 ± 10 nm.

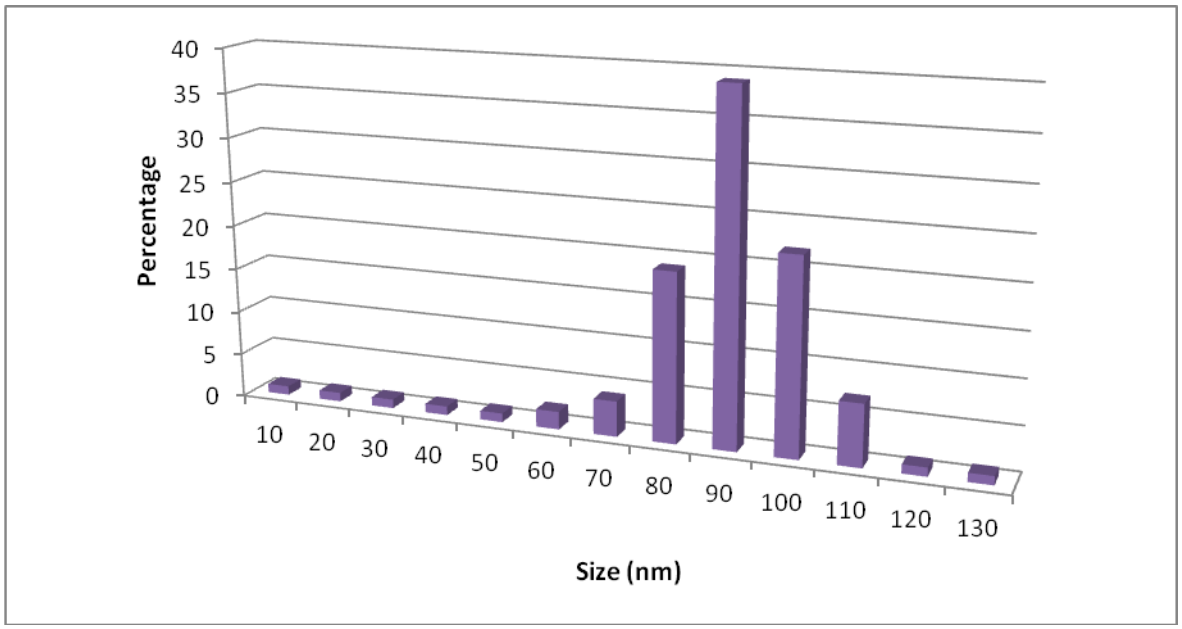

Figure 3. Size distribution of silver nanoparticles

3.3. Bioelectrochemical studies

The electrochemical cell voltage*i.e.*, the potential difference between the sensor electrode and the reference electrode (Ag/AgCl), changes with the variation in the composition of the penicillin test electrolyte solution. These conversions in the approaching potential were applied to the concentration of the penicillin in the test electrolyte solutions and the reply of penicillinase enzyme. The electrochemical answer of the penicillin biosensor depends on the measure of the catalytic activity of the penicillinase enzyme to penicillin. The hydrolysis reply of penicillin G salt in existence of penicillinase enzyme is represented in the following equation:

$$\text{Penicillin} + H_2O = \text{penicilloate}^- + H^{1+}$$

The electrochemical active surface area is one of the most important parameters to determine the catalytic characteristics of catalysts for penicillin determine, since this reaction is surface-sensitive.

As an effect of atop reaction, hydrogen ions (H^{1+}) are created and can be applied to detect the penicillin concentration [19]. Because of the generation of (H^{1+}) ions in the reaction, the pH of the solution also depletes. As the number of charges created around the silver nanoparticles based sensor electrode conversions, an alteration into the electrode potential was viewed [20]. The electrochemical evaluations applying the potentiometric method were carried out for different penicillin G salt concentrations varying from 100 μM to 100 mM made in a buffer solution at pH 7.4. The tested sensor configuration showed a wide linear dynamic range for the output response (EMF) *vs.* the logarithmic concentration of penicillin G salt solution as demonstrated in Figure 4. We acquired a slope of 135 mV/decade, which is a display of high specificity for the evaluation of penicillin.

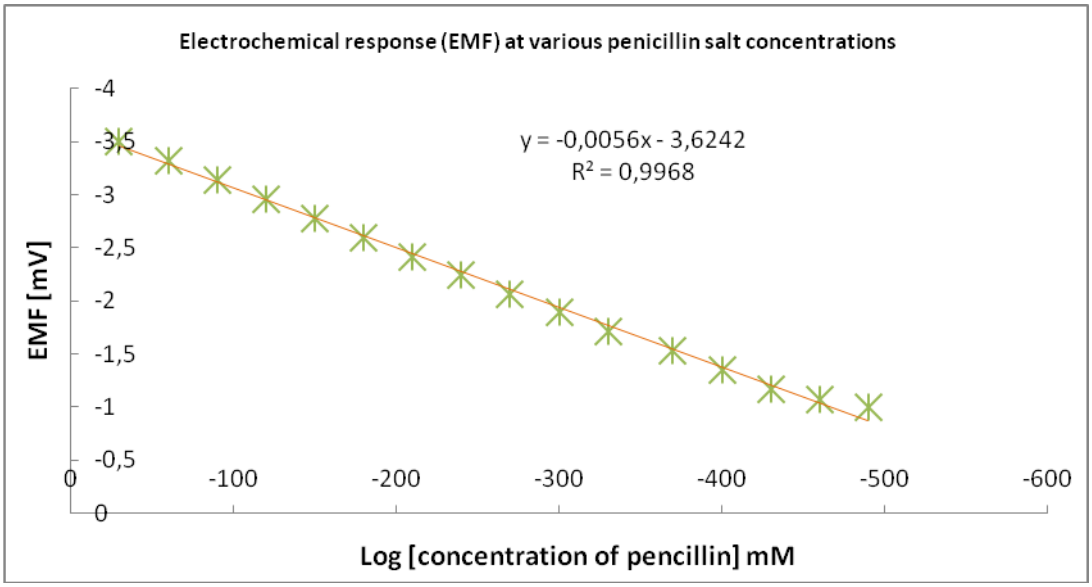

Figure 4. Calibration curve of the immobilized silver nanoparticles based sensor electrode showing the electrochemical response (EMF) at various penicillin salt concentrations (100 μM to 100 mM) with Ag/AgCl reference

3.4. Performance evaluation of the sensor

To assess the performance of a sensor it is important to evaluate different parameters such as possible concentration detection range, selectivity, detection limit, reproducibility, and response time *etc*. The reproducibility is a very notable parameter for the activity measurement of a sensor in order to recognize the consistency in acting performance. During the existing analyses, we fabricated five sensor electrodes freely applying the equal positions also immobilized the enzyme onto the silver nanoparticles in order to study the reproducibility and life time stability of the proposed sensor. To investigate the reproducibility of the given sensor, we examined the potentiometric reply of all five sensor electrodes in 5, 10, 20 and 30 mM penicillin solutions also the relative standard deviation was detected to be less than 4% as demonstrated in Figure 5.

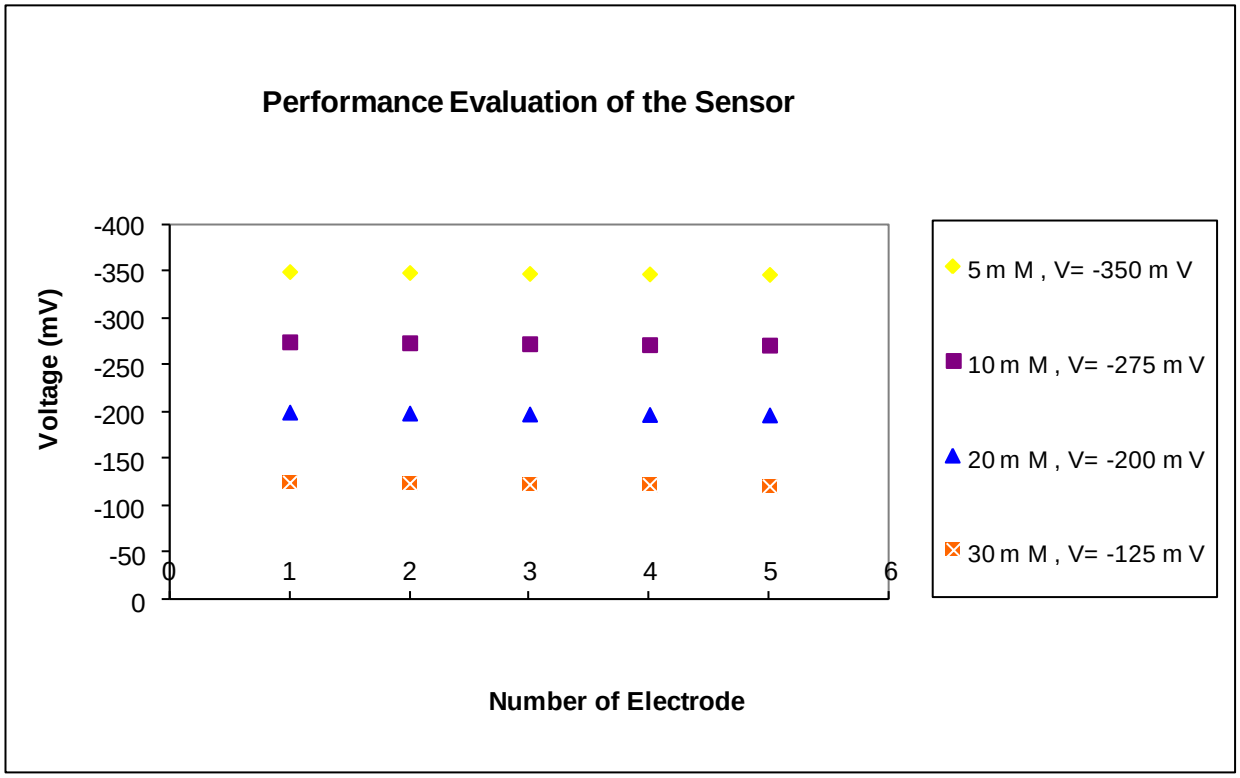

Figure 5. Performance Evaluation of the Sensor for Selective Detect of Penicillin by using of Silver nanoparticles

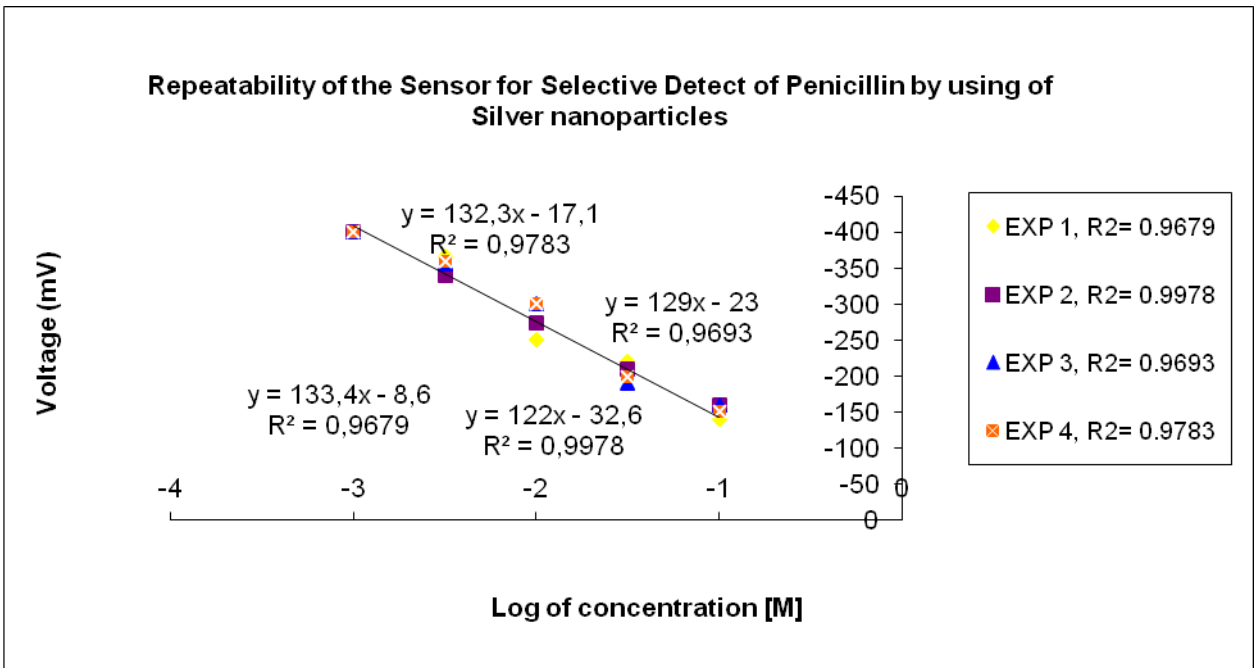

Figure 6. Calibration curves showing the biosensor repeatability at room temperature after 2–3 h passage of penicillin G salt solution from 100 μM to 100 mM concentration range.

In addition, the repeatability of the displayed sensor was also evaluated by acting four analyses with the equal sensor electrode for four sequential days, and following each calculation the electrode was soaked in a phosphate buffer solution (PBS) and later that it was dehydrated and saved at 4 °C. The sensor has demonstrated an excellent repeatability action as exhibited in Figure 6.

The effect of temperature on the performance of the sensor response was also studied by varying the temperature from 15 °C to 85 °C. The results are shown in Figure 7. During the experiments, a trend of gradual increase in the EMF answer of sensor electrode with increasing temperature was viewed, and it reached its maximum amount at around 50 °C. This is due to the reality that the enzyme has its maximum behavior at 50 °C and above 50 °C there was a sudden decrease in the EMF reply of the sensor due to the heating outcome on the immobilized enzyme, which degraded the enzyme functionality. However, the sensor demonstrated maximum reply at 50 °C but it was not as stable as at room temperature. Hence, we have elected to act at room temperature 25 ± 2 °C for the ease of exercised calculations furthermore also to avoid evaporation of the solution.

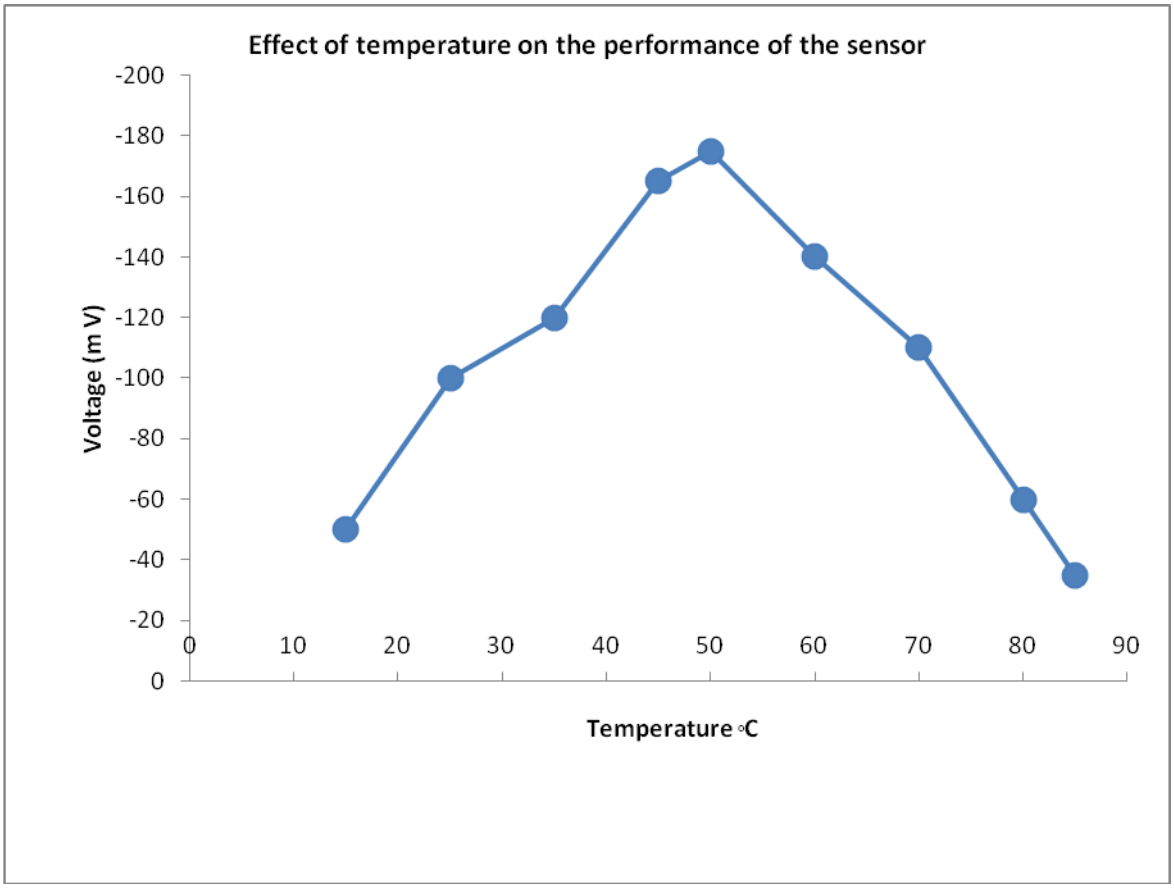

Figure 7. Effect of temperature on the performance of the sensor response

The selectivity is an important parameter for the performance evaluation of biosensors, as it gives the special affinity for viewing definite target ions in the existence of other interfering ions. The selectivity of any biosensor can be calculated in an electrolyte test solution by summing other

interfering ions. The enzyme penicillinase has adequate selectivity [21-26]. For penicillin, the enzyme-analyte reaction is highly characteristic also created the charged ions which were selectively evaluated by our proposed sensor. The penicillinase is very particular in reaction with penicillin, even in presence of other interfering species. We detected no definitive antagonist of sensor with Na^{1+}, K^{1+}, d-glucose, ascorbic acid, uric acid, urea, sucrose, lactose, glycine, penicilloic acid and cephalosporins. In Figure 8, it can be observed that on combining entire above interfering elements (100 μM of each) into 1 mM of our penicillin electrolyte solution, no conversions on the output signal stability or magnitude was viewed. Furthermore, the sensor additionally demonstrated a very fast response time of less than 4 second when the signal reached its steady state stable value.

Table 1. The time response curve of the proposed sensor in a 1,000 μM penicillin electrolytic test solution in presence of interfering species

EMF (mV)	Time [s]
-30	1
-95	2
-174	3
-175	4
-175	5
-175	6
-175	7
-175	8
-175	9
-175	10
-175	11
-175	12
-175	13
-175	14
-175	15
-175	16
-175	17
-175	18
-175	19
-175	20
-175	21
-175	22
-175	23
-175	24
-175	25
-175	26
-175	27
-175	28
-175	29
-175	30

The storage firmness of the offered sensor has been assayed with a categories of analyses acted frequently for more than four weeks and the sensor electrodes were conserved at 4 °C when not in function. It has been viewed that the sensor held their enzymatic activity up to 94% of their beginning behaviors demonstrating good storage skill and reusability for a long period of time as observed in Table 2.

Table 2. Calibration curve showing the study of the electromotive (EMF) response with the influence of storage at 4 °C for three weeks

EMF (mV)	Number of days
-175	1
-174.5	2
-174	3
-173.5	4
-173	5
-172.5	6
-172	7
-171.5	8
-171	9
-170.5	10
-170	11
-169.5	12
-169	13
-168.5	14
-168	15
-167.5	16
-167	17
-166.5	18
-166	19
-165.5	20
-165	21

4. CONCLUSION

Nanoelectrochemistry performs definitive responsibility in the fabrication of various sensors, and devices for detecting molecules at very low concentrations. In this study sensor electrodes were prepared after the immobilization of enzyme penicillinase on to the surface of grown silver nanoparticles in combination with (ANB-NOS) as a cross linker. After fabricate a good and exact sensor, the effect of temperature on the performance of the sensor response was also studied by varying the temperature from 15 °C to 85 °C. During the experiments, a trend of gradual increase in the EMF answer of sensor electrode with increasing temperature was viewed, and it reached its maximum amount at around 50 °C. For penicillin, the enzyme-analyte reaction is highly characteristic also created the charged ions which were selectively evaluated by our proposed sensor. The

penicillinase is very particular in reaction with penicillin, even in presence of other interfering species. We detected no definitive antagonist of sensor with Na^{1+}, K^{1+}, d-glucose, ascorbic acid, uric acid, urea, sucrose, lactose, glycine, penicilloic acid and cephalosporins.

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