Bioprocess and Biosystems Engineering
https://doi.org/10.1007/s00449-021-02618-3

RESEARCH PAPER

Enzyme-based sensing on nanohybrid film coated over FTO electrode for highly sensitive detection of antibiotics

Nidhi Chauhan[1] · Sapna Balayan[1] · Shaivya Gupta[1] · Jaskaran Singh[2] · Utkarsh Jain[1]

Received: 10 March 2021 / Accepted: 27 July 2021

Abstract

Cefepime and Meropenem are the new class of antibiotics, which are particularly used as last potent defender or the antibiotics of the last resort against multi-resistant bacterial species. In this paper, an impedance-based electrochemical biosensor was fabricated for identifying antibiotics of last resort in the forensic samples including gastric lavage and other body fluids. The sensor was developed using platinum nanoparticles (PtNPs) and electrodeposited zinc oxide- zinc hexacyanoferrate hybrid film (ZnO/ZnHCF) on the surface of a fluorine-doped glass electrode (FTO). Further, penicillinase was immobilized onto the modified electrode using penicillinase enzyme. The developed biosensor exhibits a good analytical response for the detection of antibiotics evaluated using electrochemistry studies. The linear response of the fabricated electrode was observed from 0.1 to 750 µM and the electrode limit of detection (LOD) was observed as 0.1 µM. The sensor confirms good accuracy, is highly selective, and sensitive for the target. While storing the modified electrode at 4 °C, the stability of biosensor was evaluated for 45 days, and activity loss of 30–40% was observed. The highly sensitive interface of Penicillinase@CHIT/PtNP-ZnO/ZnHCF/FTO electrode shows a promising future in forensic studies.

Keywords Cefepime · Meropenem · Platinum nanoparticles · Penicillinase

Introduction

Antibiotic resistance is a slow and lethargic crisis. According to World Health Organization (WHO), antibiotic resistance causes more than 700,000 deaths per year globally [1–3]. Over the past few years, bacteria have evolved resistance against the drugs which have been used to act against deadly infections [4]. There are several factors that are associated with antibiotic resistance in respect to transmission, dissemination, and the emergence of bacterial species (i) environmental conditions of resistant causing bacteria, (ii) evolution of resistance in bacterial competence, (iii) specific characteristics of antibacterial drugs contributing in global dissemination, (iv) bacterial properties responsible for the rate of resistance, (v) role of host-associated factors (hygiene, vaccination, population density, healthcare, and travel) which are needed to be resolved in the future [5,6].

Cefepime and meropenem are contemplated as a fundamental critical part of our anti-microbial armamentarium. From a large number of β-lactams, the prominent strength against Gram-positive, and Gram-negative microorganisms was observed in carbapenems [4]. Accordingly, once a patient is associated with harbouring resistant microorganisms or is fatally sick, they are regularly utilized as "last-line agents" or "antibiotics of last resort" [7,8]. Cefepime and Meropenem are the new class of antibiotics that are flexible and particularly used as last strong safeguard against multi-resistant bacterial sepsis [9]. For the peptidase domain of penicillin-binding proteins (PBPs), the Carbapenems act as mechanism-based inhibitors (MBIs) and can inhibit peptide cross-linking and peptidase response to proteins. Besides, in certain conditions Carbapenems acts as slow substrates or inhibitors of β-lactamases and targeting PBPs as they have a capacity of binding with multiple PBPs. In Gram-negative bacteria, the repression of PBPs during autolysis process results in the weakening of glycan backbone and thereby damage the cell due to osmotic pressure [4].

✉ Utkarsh Jain
karshjain@gmail.com; ujain@amity.edu

[1] Amity Institute of Nanotechnology (AINT), Amity University Uttar Pradesh (AUUP), Noida 201313, India

[2] School of Allied Health Sciences (SAHS), Sharda University, Greater Noida 201306, Uttar Pradesh, India

Published online: 12 August 2021

Presently, several methods for testing antibiotics are being practiced in laboratories. They are dilution method, disk diffusion method, mechanism-specific tests, genotypic methods, and screening method [10]. A study for curcumin identification was conducted based on the agar dilution method. Curcumin stock solution was prepared in a dimethyl sulfoxide (128 mg/L) that was incorporated for the development of Mueller–Hinton agar (MHA) plates while increasing curcumin concentrations [11]. The development of a TDtest with modification in disk diffusion method for detecting bacterial growth in inhibition zone while diffusing the antibiotics was reported. The test is used to detect antibiotics that can eliminate the effect of tolerant bacteria [12]. Furthermore, Fourier-Transform Infrared (FTIR) spectroscopy-based metabolic fingerprint mechanism-specific testing was developed for high-throughput whole-cell screening. In this reported study, the *Escherichia coli* metabolism was incorporated for metabolic fingerprint detection evaluated by 15 antibiotics [13]. Moreover, the detection of antimicrobial resistance of *Helicobacter pylori* using genotypic and phenotypic detection methods were performed. The study reported resistance for clarithromycin, levofloxacin, and metronidazole [14].

However, these methods suffer drawback as they are very tedious and consumes a lot of time. Recent studies describe the development of various platforms for antibiotic detection [15] such as ultrasensitive development of an aptasensor for identification of kanamycin (Kana) antibiotic. The electrode was first modified with hairpin probe 2 (HP2) which was covalently conjugated onto the surface of electrode and then the targeted kana was slowly dropwise added on the modified surface. The further modification of strand-displacement reaction and single-stranded DNA (S1) trigger hybridization chain reaction carried out on the electrode. The sensor facilitates the detection of Kana in milk and provides higher sensitivity and selectivity [16]. Another study shows Kana detection with glutathione-loaded liposome-encoded magnetic beads where the process is initiated by palindromic fragment-mediated single-chain amplification (PFMSCA). The system is focused on the development of one oligonucleotide (hairpin HP_1) consisting of template, primer, and recognition element for processing magnetic separation, target recognition, and amplification [17]. Besides, a Z-scheme photoelectrochemical (PEC) biosensing platform has been developed based on a palindromic molecular beacon (PMB) for the detection of Kana. The platform was modified with gold nanoparticles and bismuth oxychloride (BiOCl-Au) for obtaining enhanced photocurrent [18]. A dual-detection platform was developed for Kana determination with a spectrofluorometer which can be seen via naked-eye. The system is based upon two sequential processes: target-aptamer binding and hybridization chain reaction. The platform exhibits a dynamic linear range from 0.002 to 5 nM and the detection limit of the platform was very low (1.2 pM) [19].

The electrode materials integrated for developing a biosensor need to show excellent properties such as active sites, excellent carriers to avoid hindrance of the electron transfer, large surface area for binding with the biological molecules, forensic samples, and electrode materials. Recent studies show that the bimetallic nanoparticles contribute excellent optical, electronic, and catalytic properties towards the development of a biosensing platform. These materials constitute two distinct metal elements and in a result provide synergistic effect [20–22]. A study describes the development of a platform incorporated with bimetallic iron/nickel (Fe/Ni) at a nanoscale for eliminating β-lactam antibiotics. The active sites of Fe oxide assist adsorption and electron degradation at the surface of Zero-valent Fe NPs (nZVI). The sensing platform describes the removal of antibiotics with simultaneous adsorption and desorption processes [23]. In another study, removal of tetracyclines (TCs) was performed using an nZVI modified platform coated with graphene oxide (GO) and copper (Cu) bimetallic nanoparticles. The obtained results show the efficacy of TCs which were enhanced in the case of Cu nano-adsorbents [24]. Cefotaxime was removed from an aqueous solution using a bimetallic nanoparticle-based adsorbent cobalt/iron (Co/Fe) loaded with alkali modified biochar (Co/Fe/MB). Due to the combined effect of Co/Fe/MB the higher efficiency (99.23%) for cefotaxime removal was obtained [25]. Another study describes the removal of amoxicillin within 30 min evaluated using carbon nanofibers (CNFs) modified with cobalt/silver (Co/Ag) bimetallic nanoparticles (Co@CNFs-Ag). The processed system facilitates an effective oxidation and heterogeneous activation of peroxymonosulfate [26]. Therefore, owing to the excellent electrochemical properties, the bimetallic nanoparticles for electrode modification were incorporated in this study.

Platinum nanoparticles (PtNPs) are newly emerging electrode materials in biosensing applications. PtNPs have exceptional characteristics such as large surface areas with enhanced charge transfer and show magnificent amperometric and impedimetric properties of sensors over a broad range of concentrations [27–34]. On the other hand, zinc oxide nanoparticles (ZnONPs) are biocompatible, chemically stable, show good electronic behaviour, possess better metallic, and structural properties [35]. Zinc oxide nanoparticles provide direct charge transfer over enzyme immobilization and facilitate retention of the biological activity on the electrode surface [36,37]. Once the electrode is modified with Pt and ZnO nanoparticles, it provides a synergistic effect on the surface of the fabricated electrode.

In this study, an impedimetric sensor was constructed for the detection of antibiotics of the last resort, cefepime in the forensic samples. The biosensor is developed using

bimetallic nanoparticles (PtNPs and ZnONPs) electrochemically adsorbed by deposited zinc hexacyanoferrate (ZnHCF) nanohybrid film on the surface of a fluorine-doped glass electrode. Further, penicillinase was immobilized onto the moderated electrode using the penicillinase enzyme. The principle of the developed biosensor is based upon the transfer of electrons between the fabricated electrode and biomolecule.

Materials and designing processes

Chemicals and reagents

Cefepime (200 mg) was procured through Indian Pharmacopoeia Commission. Penicillinase enzyme and Bovine serum albumin (BSA), Fluorine-doped tin oxide (FTO) electrode were procured from a U.S. based company, Sigma Aldrich. Hexachloroplatinic (IV) acid hydrate ($H_2PtCl_6{\cdot}xH_2O$), Silver nitrate ($AgNO_3$), Polyvinyl pyrrolidone (PVP), Ethylene glycol (EG), Sulfuric acid (H_2SO_4), Ferrocyanide, Ferricyanide $[Fe(CN)_6]^{3-/4-}$, Chitosan (CHIT) and Glutaraldehyde (GA), Potassium chloride (KCl), Zinc nitrate ($Zn(NO_3)_2$) and Potassium hydroxide (KOH) were acquired from a research laboratory in India "Sisco Research Laboratory", India. Gastric lavage was artificially made by mixing a few drops of normal saline, ammonia, and citric acid in water. Distilled water (DW) was used throughout the experiments.

The moderated electrode was stepwise characterized using Scanning electron microscopy (SEM). Further, the electrode was characterized with electrochemical measurements after every modification stage including electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) techniques through Biologics Potentiostat (Biologics-SP150, EC-lab).

Pre-treatment of the FTO electrode

Prior to modification, the electrode was first rinsed with methanol in an ultrasonic bath to remove the unwanted impurities. Afterward, the electrode was dried and immersed in a 10^{-3} mol dm^{-3} solution of methanol and *N*-3-(Trimethoxysilyl)propyl-*N*,*N*,*N*-trimethylammonium chloride (TMATMS) or *N*-3-(Trimethoxysilyl)propyl-*N*-octadecyl-N,Ndimethylammonium chloride (ODMATMS) for 24 h. The electrode was washed, dried, and was submerged in an aqueous solution of porphine or ferrozine for 15 min. After washing and drying, the functionalized FTO electrode was treated for 15 min with metal cations solution, iron (Fe^{2+}), and copper (Cu^{2+}) perchlorates, respectively. For further electrochemical studies, the electrode was finally cleansed with DW and dried at room temperature [38].

Synthesis of platinum nanoparticles

The PtNPs were synthesized by precipitation method. A solution was prepared with 2×10^{-3} M $AgNO_3$ (0.5 mL) in EG while abruptly adding 0.375 M PVP (3 mL), and 0.00625 M hexachloroplatinic acid (1.5 mL) was then added dropwise in the solution for 15 min. The compound formed was refluxed for 5 min following centrifugation. The suspension was separated using acetone to get precipitate and supernatant. Further, the obtained precipitate was washed two times with ethanol and further dispersed in it [39].

Synthesis of zinc nanoparticles

The ZnONPs were synthesized by direct precipitation method, using precursors $Zn(NO_3)_2$ and KOH. A solution was prepared with 0.2 M zinc nitrate in DW, the prepared KOH solution of 0.4 M was added into the zinc nitrate solution with continuous stirring at room temperature. The white suspension was obtained after the chemical reaction was separated using centrifugation (5000 rpm for 20 min) and the washing of the product was carried out with DW for three times. The final rinse was done with ethanol. The obtained product was calcinated at 500 °C for 3 h [40].

Stepwise assembly of the FTO electrode

Electrode modification with ZnO/ZnHCF hybrid film

The pre-treated electrode was modified with ZnO/ZnHCF hybrid film on the surface of FTO through an electrochemical reaction. The films were deposited on the surface of the FTO electrode by cyclic voltammetry (CV) within a potential ranging from + 0.4 V to + 1.6 V at a constant scan rate for 30 cycles in a solution of $Zn(NO_3)_2$, $[Fe(CN)_6]^{3-/4-}$ (5 mM)/KCl (0.1 M) and H_2SO_4 (0.1 M maintaining pH 2.0). Further, the electrode was washed with DW.

Electrodeposition of PtNPs on modified ZnO/ZnHCF/FTO electrode

The electrochemical deposition of PtNPs was conducted in an electrochemical cell system constituting three electrodes as working, auxiliary (Pt), and reference (Ag). The deposition of PtNPs was performed by CV applying an amplitude from 0.4 to 1.6 V for ten cycles in a $[Fe(CN)_6]^{3-/4-}$/KCl mediator solution.

Modification of electrode with chitosan

The CHIT solution (1% w/v) was prepared in 5 mL (v/v) DW and kept for ultrasonication for 1 h followed by vortexing. The prepared suspension was then drop casted (5 mL) onto the modified PtNPs-ZnO/ZnHCF/FTO electrode. Further, the electrode was dried at room temperature for 12 h.

Immobilization of the penicillinase enzyme

Penicillinase enzyme was immobilized on the modified CHIT/PtNPs-ZnO/ZnHCF/FTO electrode through GA coupling, 5 mL of GA (2.5%) was first allowed to deposit over chitosan. Then a mixture of BSA (0.5 mg/mL) and penicillinase (30 U/mL) were prepared in phosphate buffer (pH 7.0). From the prepared mixture, 5 µL was placed onto CHIT/PtNPs-ZnO/ZnHCF/FTO modified electrode. The modified electrode was kept overnight at 4 °C for drying to achieve the cross-linking reaction. Afterward, the electrode was used for analysis [41].

Sample preparation for cefepime

For validating the fabricated electrode, the real sample obtained from the laboratory was prepared according to the described method. Each 500 mg of forensic sample (gastric lavage and whole blood) was dissolved in a small amount of acetone, centrifuged for 10 min, and then was filtered. The clear filtrate was further used for experiments. A stock solution was prepared for each drug by following a standard procedure through diluting it with DW to 1000 ppm and obtained real samples were stored under − 80 °C to prevent it from degradation and impurities. The samples were collected by diluting stock solutions (from 1000 ppm to 1 ppb) with diluted gastric juice for sensing measurements [42].

Electrochemical performance with the substrate on Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode

The electrochemical measurements for the developed biosensor were conducted on BioLogic potentiostat SP150. The electrochemical system represents three electrodes configuration Ag/AgCl (reference), Platinum (auxiliary), and Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode (working).

The CV measurements were performed in a potential ranging from − 0.8 to 1.0 V at a constant scan rate for stepwise surface modification of the electrode. The activity of the biosensor was measured with CV under various concentrations of cefepime ranging from 0.1 to 750 µM.

Stability and reproducibility of Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode

The reusability of modified Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode was evaluated after washing with a solution of 7.0 pH sodium phosphate buffer (0.1 M). The modified electrode was stored at 4 °C and storage stability was checked after 2 months. The activity of Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode was performed every week.

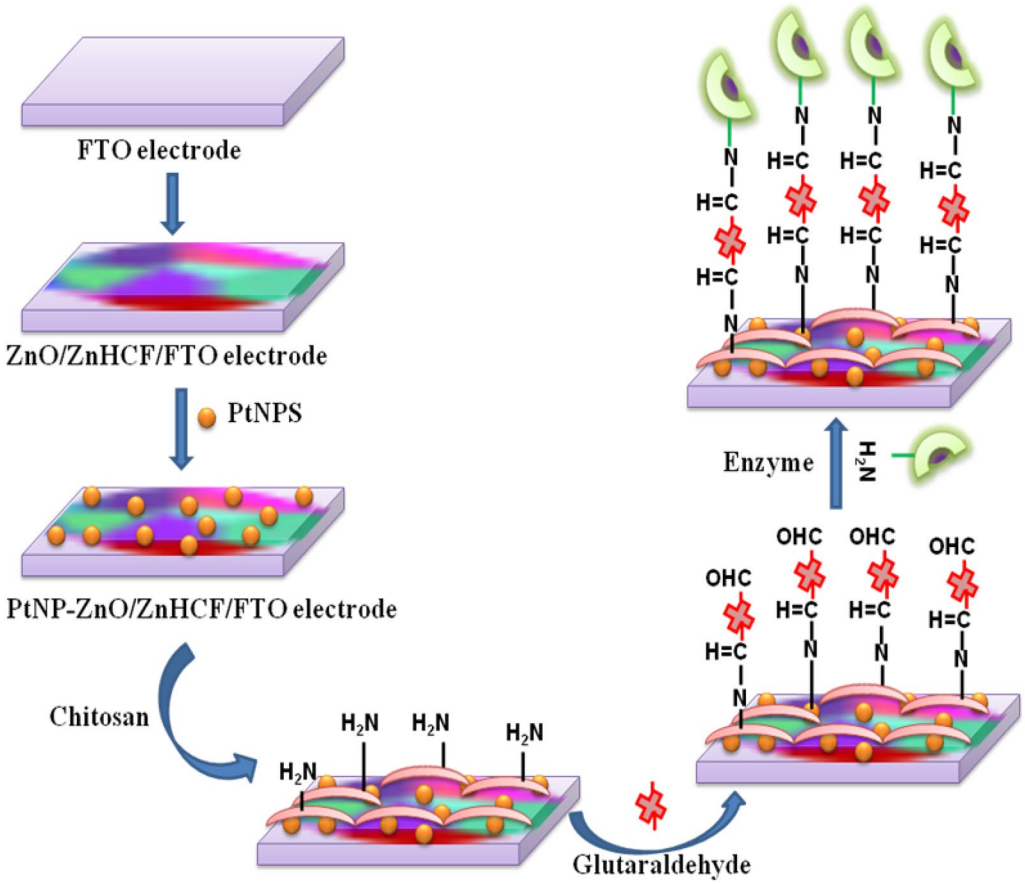
Electrode performance and sensing scheme

The electrode was modified with nanomaterials (PtNPs-ZnO/ZnHCF/FTO) to obtain enhancement in electrochemical signals caused due to electron transfer during redox reaction and high biocompatibility. Further, the electrode was immobilized with penicillinase enzyme and a low potential is applied on the electrode which facilitates an enzymatic reaction occurred due to the reduction of cefepime on electrode surface. The electrochemical oxidation and reduction peaks were observed on the electrode in a mixture of $[Fe(CN)_6]^{3-/4-}$ (5 mM) and KCl (0.1 M) solution. The produced current was measured with electrochemical techniques (Scheme 1).

Results and discussion

Stepwise surface characterization of developed nano-interface

The surface morphology of the sensor was observed at every stage with the Scanning electron microscopy (SEM), performed at AIARS, Amity University Uttar Pradesh (AUUP), Noida, India. Figure 1A shows the uniform and smooth surface of the bare FTO electrode. Figure 1B illustrates the surface imagining of a ZnONPs on ZnHCF/FTO electrode. The image clearly depicts the uniformly distributed rod shaped structures on the surface indicating the presence of ZnONPs. Figure 1C represents the morphology of an electrode modified with PtNPs. The homogenous spherical structure resembles the presence of PtNPs on the ZnO/ZnHCF/FTO electrode surface. Moreover, the presence of globular morphology in Fig. 1D displays the immobilization of penicillinase enzyme on the modified CHIT/PtNPs-ZnO/ZnHCF/FTO electrode surface.



Scheme 1 Schematic steps to fabricate an enzyme immobilized PtNPs-ZnO/ZnHCF modified fluorine-doped glass electrode

Electrochemical measurements of the modified Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode

The modified electrode was stepwise characterized by CV. Figure 2 illustrates the oxidation and reduction for the FTO electrode (bare), deposition of ZnONPs and PtNPs, and further modification with penicillinase enzyme in the presence of $[Fe(CN)_6]^{3-/4-}$/KCl mediator solution at a constant scan rate of 20 mV/s. The CV measurement of the bare FTO electrode shows no defined oxidation and reduction peak (curve i). In the next step, the FTO electrode was modified with ZnONPs (curve ii) hence the oxidation peak was observed at 0.58 mA. Further, the electrode was modified with PtNPs (curve iii) and a higher oxidation peak was observed at 1.15 mA. PtNPs acts as a catalyst and enhances the electrode conductivity promoting a fast electron transfer. Additionally, the synergistic effect of the nanoparticles provides a large surface area. Finally, the nanoparticle-coated electrode was modified with penicillinase enzyme (curve iv), the oxidation peak was obtained at 0.46 mA. Non-insulating characteristics of the enzymes resulting in a decrease of the current value while adding enzyme on the PtNPs-ZnO/ZnHCF/FTO modified electrode.

Electrochemical impedance spectroscopy for Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode

EIS measurements were performed for studying the internal resistance of the system [43]. The EIS measurements were conducted using three electrodes system. In EIS, the electron transfer resistance (Rct) value is determined by the semicircle diameter whereas the linear part represents the diffusion in the system. Figure 3 represents the Nyquist plot for the stepwise modification of the fabricated Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode. The Nyquist plot describes that the highest Rct value was observed with bare FTO electrode whereas it gradually decreases while modifying with ZnONPs and further decreases with PtNPs/ZnONPs modification. It was observed that after loading the enzyme onto the modified PtNPs-ZnO/ZnHCF/FTO electrode the Rct value is increased. The lower Rct value was obtained for the fabricated electrode as compared to the bare FTO

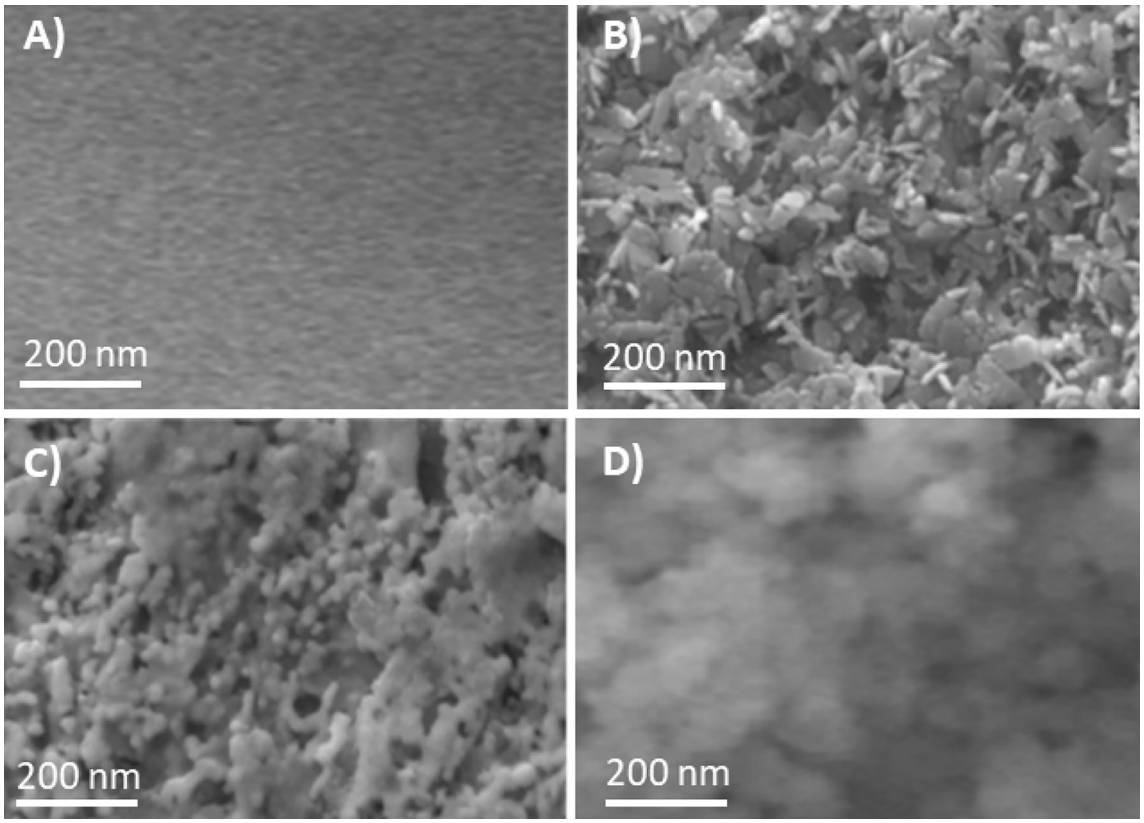


Fig. 1 Surface morphological studies by SEM image of the (**A**) bare FTO electrode, (**B**) ZnO/ZnHCF/FTO electrode, (**C**) PtNPs-ZnO/ZnHCF/FTO electrode, (**D**) Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode

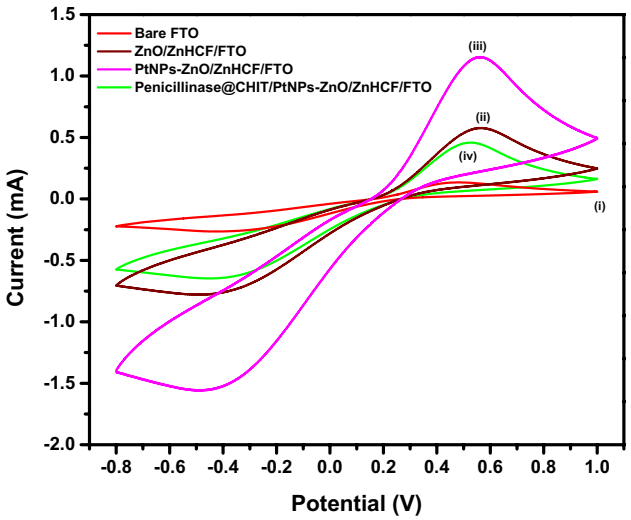


Fig. 2 Biosensing response studies (CV) of (i) bare FTO electrode, (ii) ZnO/ZnHCF/FTO electrode, (iii) PtNPs-ZnO/ZnHCF/FTO electrode and (iv) Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode in $[Fe(CN)_6]^{3-/4-}$ (5 mM) and KCl (0.1 M) electrolyte solution

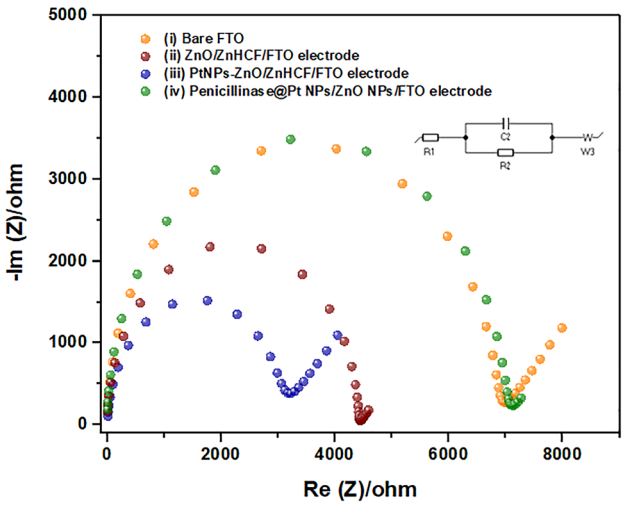


Fig. 3 The electrochemical impedance spectroscopy of (i) bare FTO electrode, (ii) ZnO/ZnHCF/FTO electrode, (iii) PtNPs-ZnO/ZnHCF/FTO electrode and (iv) Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode in electrolyte solution $[Fe(CN)_6]^{3-/4-}$ (5 mM) and KCl (0.1 M). Frequency range of 0.01–10 kHz

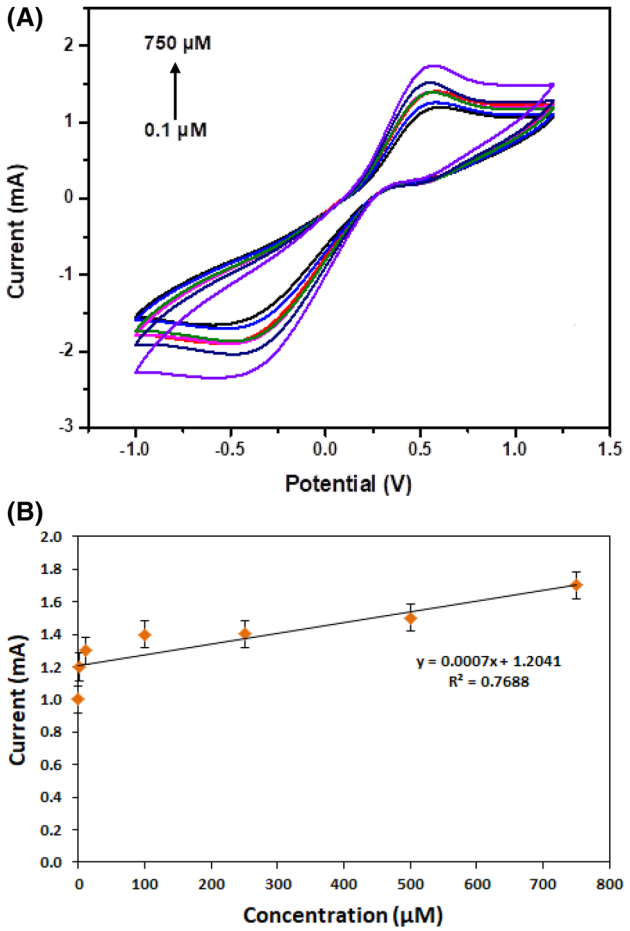


Fig. 4 **A** Response study of Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode in $[Fe(CN)_6]^{3-/4-}$ (5 mM) and KCl (0.1 M) electrolyte solution with different concentration of Cefepime antibiotic. **B** Calibration Plot on the basis of response studies of Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode in $[Fe(CN)_6]^{3-/4-}$ (5 mM) and KCl (0.1 M) solution with different concentration of Cefepime antibiotic

electrode because the enzyme restricts the transfer of electrons in the system. These results corroborate the CV and EIS results.

Electro-catalytic characteristics of the fabricated sensor with various concentrations

Cefepime detection was performed on a developed biosensor with variable concentrations. The fabricated electrode was dipped in a different concentration solution (0.1 to 750 µM). CV response was obtained for these concentrations as illustrated in Fig. 4A. A gradual enhancement in the peak current was obtained with increasing concentration indicating adsorption of antibiotics on the electrode surface causing an enhanced electron charge transfer at higher concentrations. A calibration curve was plotted between concentration vs higher oxidation peak current for each concentration as illustrated in Fig. 4B. The calibration curve describes the linear range of the developed biosensor from 0.1 to 750 µM with regression equation of $y = 0.0007x + 1.2041$ and regression coefficient (R^2) 0.7688 where concentration is in µM and peaks current in mA. The sensitivity of the fabricated biosensor was calculated as 0.862 mA/µM and the detection limit of the biosensor was observed as 0.1 µM.

Optimization of the modified Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode

To determine the optimum pH for the fabricated biosensor, CV was performed in a $[Fe(CN)_6]^{3-/4-}$/KCl electrolyte solution at potential ranging from − 1.0 to 1.0 V under various pH values from 6 to 7.6 (6, 6.4, 6.8, 7.2, 7.6). Figure 5A graph is plotted for various pH ranges showing an enhancement in the oxidation and reduction peaks with a gradual increase in the pH value. Higher oxidation and reduction peak were observed with pH 7.6, therefore it was considered as optimum pH for the fabricated biosensor.

Similarly, to study the optimum temperature for the developed biosensor, measurements through CV were performed in an electrolyte solution $[Fe(CN)_6]^{3-/4-}$/KCl containing 750 µM cefepime, with incubation temperature ranging from 20 to 40 °C at an interval of 5 °C. Figure 5B represents the gradual increase in the oxidation and reduction peak while increasing temperature. A higher oxidation peak was observed at 40 °C, therefore, 40 °C temperature was selected for further studies.

Interference studies for the fabricated biosensor

The fabricated Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO biosensor was evaluated in several interferents such as Amphetamine, Ketamine, Ephedrine, Thebaine, Methamphetamine, and Codeine as shown in Fig. 6A. The biosensor shows 5% activity loss in the case of Amphetamine, 20% activity loss for Ketamine, 25% for Ephedrine, 16% in the case of Thebaine, 26% and 10% for Methamphetamine, and Codeine, respectively.

Stability, accuracy, and reproducibility of the developed biosensor

The shelf-life of Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO fabricated biosensor was examined by performing CV measurements with 750 µM cefepime antibiotic concentration in a $[Fe(CN)_6]^{3-/4-}$/KCl electrolyte solution every week.

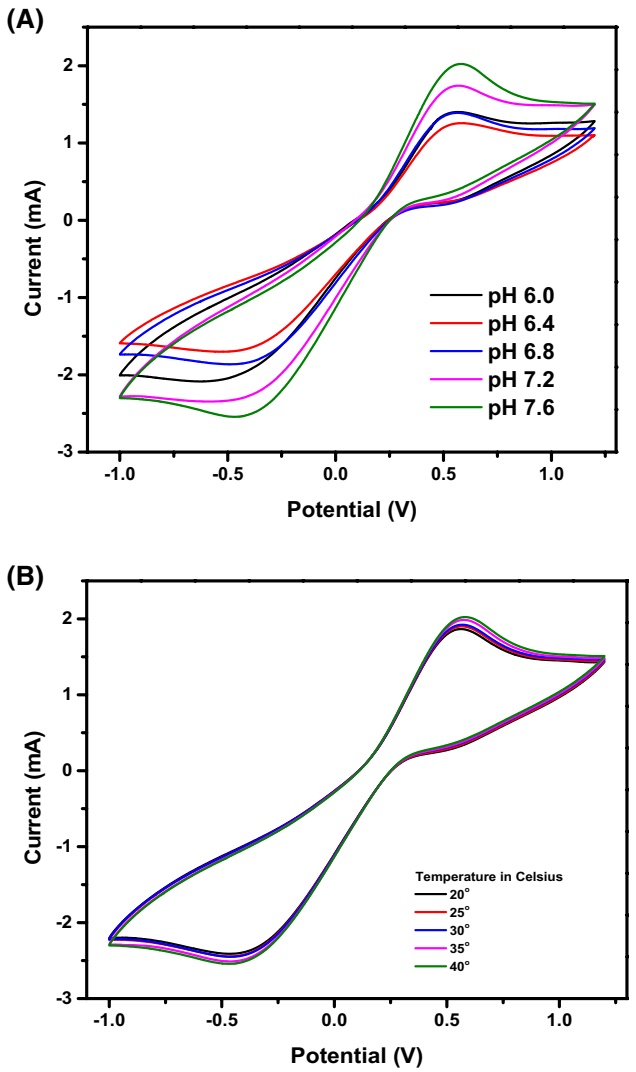


Fig. 5 (**A**) Effect of different pH ranging from 6 to 7.6 with an interval of 0.4 on Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode in $[Fe(CN)_6]^{3-/4-}$ (5 mM) and KCl (0.1 M) electrolyte solution. (**B**) Effect of different temperature ranging from 20 to 40 °C with an interval of 5 °C on Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode in $[Fe(CN)_6]^{3-/4-}$ (5 mM) and KCl (0.1 M) solution

It is observed that the fabricated electrode keeps hold of the activity up to 60–70% up to 6–7 weeks and stored at 4 °C. The loss in activity of bioelectrode was determined by the denaturation of the enzyme. The reproducibility of the sensor was observed with five identically prepared electrodes.

Figure 6B illustrates the reproducibility of the fabricated biosensor based on the electrochemical current measurement for the five identical electrodes maintaining the same conditions. Table 1 shows the accuracy of fabricated biosensor with seven different concentrations of cefepime in gastric lavage samples procured from the laboratory. The accuracy of the developed biosensor was observed within the range of 90% to 99.6%.

Application of the proposed biosensor in gastric lavage

Gastric Lavage was collected from Bio diagnostic Lab, Rohini, New Delhi for the evaluation of cefepime antibiotic on Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode. The modified electrode was examined five times repeatedly in the received human serum samples. The sensor shows high sensitivity for cefepime detection in the biological samples.

Conclusion

A novel biosensing platform for monitoring of antibiotics in forensic samples through impedimetric-based sensing on FTO electrode was developed. To modify the working electrode, the combination of novel nanocomposite was utilized facilitating an increase of surface area and thereby enhancing the rate of electron charge transfer on the fabricated electrode. The PtNPs and ZnONPs were deposited on the FTO glass electrode modified with electrodeposited ZnHCF nanohybrid film. Further, the penicillinase enzyme was immobilized onto the modified electrode. It was observed that the analytical response of the developed biosensor was magnificent towards the identification of antibiotics of the last resort with an electrochemical method in the forensic samples and the biosensor operates within a wide detection range (0.1–750 μM) and at a lower potential (+0.4 V). The developed biosensor is highly sensitive (0.862 mA/μM) and it shows lower limit of detection (0.1 μM). The present electrode shows a shelf life of about 45 days. This Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO electrode once miniaturized can be utilized in the diagnosis of antibiotics of the last resort.

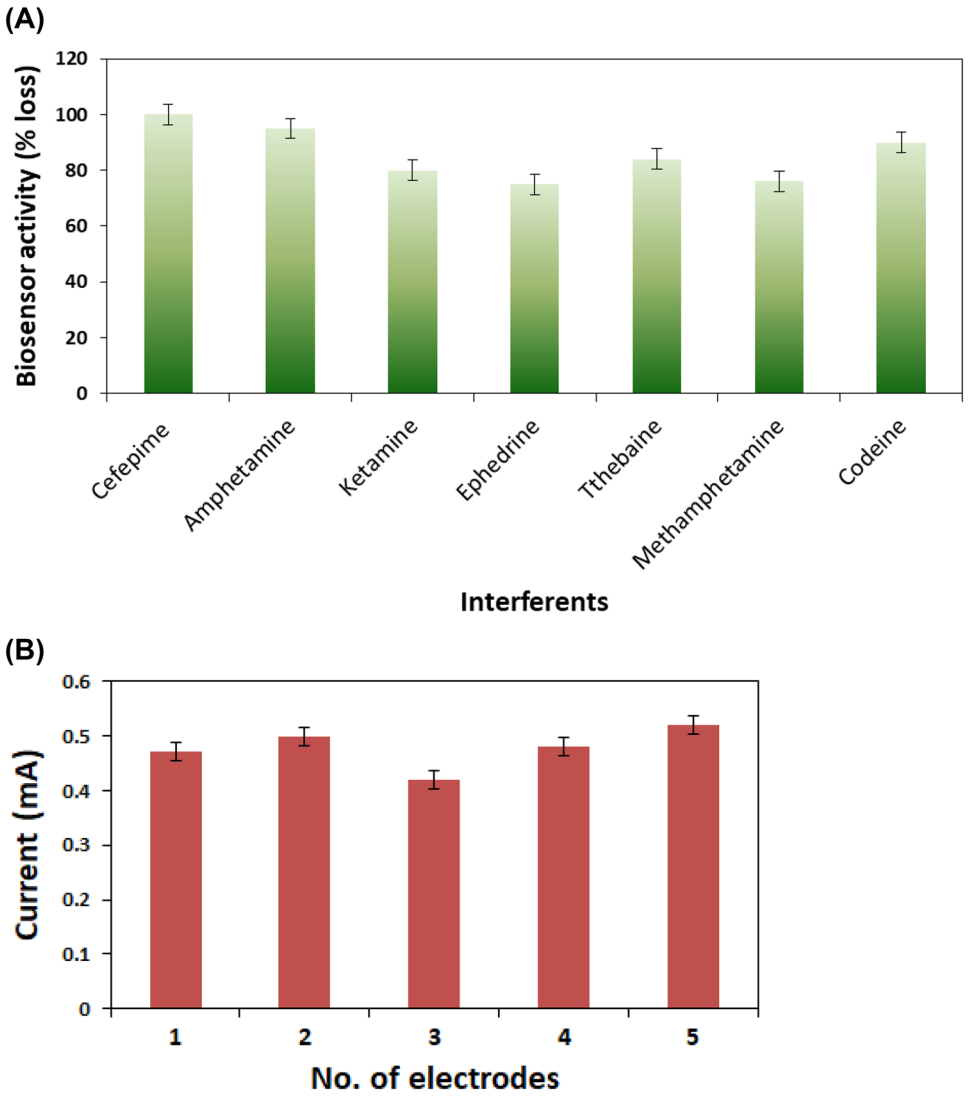


Fig. 6 (**A**) Interference study on the developed Penicillinase@CHIT/PtNPs-ZnO/ZnHCF/FTO biosensor with different interferents. (**B**) Reproducibility studies conducted for the biosensor with five identical electrodes

Table 1 Cefepime detection in gastric lavage

S. no.	Age/gender of the dead body	Cefepime concentration (μM) added	Concentration of gastric lavage (μM) through developed biosensor	Accuracy (%)
1	38/F	0.1	0.09	90.00
2	61/M	1	1.05	95.24
3	44/M	10	11	90.91
4	49/F	100	98	98.00
5	53/M	250	252	99.21
6	40/F	500	502	99.60
7	57/M	750	746	99.47

Acknowledgements This work is financially supported by Extramural Research Grant (File No. EMR/2016/007564) and Young Scientist Scheme (File No. YSS/2015/000023) of Science and Engineering Research Board, Government of India, and Technology Development Program (TDP) (TDP/BDTD/33/2019) of Department of Science and Technology (DST), Government of India and Biotechnology Industry Research Assistance Council (BIRAC) (File No. BT/IIPME0211/02/16).

Declarations

Conflict of interest The authors declare there is no financial or personal conflict of interest that may affect the presented work.

References

1. World Health Organization (2014) Antimicrobial resistance: global report on surveillance. World Health Organization
2. O'neill J (2014) Antimicrobial resistance. Tackling a crisis for the health and wealth of nations
3. Caniça M, Manageiro V, Abriouel H, Moran-Gilad J, Franz CM (2019) Antibiotic resistance in foodborne bacteria. Trends Food Sci Technol 84:41–44
4. Codjoe FS, Donkor ES (2018) Carbapenem resistance: a review. Med Sci 6:1
5. Andersson DI, Balaban NQ, Baquero F, Courvalin P, Glaser P, Gophna U, Kishony R, Molin S, Tønjum T (2020) Antibiotic resistance: turning evolutionary principles into clinical reality. FEMS Microbiol Rev 44:171–188
6. Hernando-Amado S, Coque TM, Baquero F, Martínez JL (2019) Defining and combating antibiotic resistance from one health and global health perspectives. Nat Microbiol 4:1432–1442
7. Dhingra S, Rahman NA, Peile MR, Sartelli M, Hassali MA, Islam T, Islam S, Haque M (2020) Microbial resistance movements: an overview of global public health threats posed by antimicrobial resistance, and how best to counter. Front Public Health 8:535668
8. Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VH, Takebayashi Y, Spencer J (2019) β-lactamases and β-lactamase inhibitors in the 21st century. J Mol Biol 431:3472–3500
9. Wright PM, Seiple IB, Myers AG (2014) The evolving role of chemical synthesis in antibacterial drug discovery. Angew Chem Int Ed 53:8840–8869
10. Van Belkum A, Burnham CA, Rossen JW, Mallard F, Rochas O, Dunne WM (2020) Innovative and rapid antimicrobial susceptibility testing systems. Nat Rev Microbiol 18:299–311
11. Kali A, Bhuvaneshwar PD, Charles MV, Seetha KS (2016) Antibacterial synergy of curcumin with antibiotics against biofilm producing clinical bacterial isolates. J Basic Clin Pharm 7:93
12. Gefen O, Chekol B, Strahilevitz J, Balaban NQ (2017) TDtest: easy detection of bacterial tolerance and persistence in clinical isolates by a modified disk-diffusion assay. Sci Rep 7:1–9
13. da Cunha BR, Fonseca LP, Calado CR (2020) Metabolic fingerprinting with Fourier-transform infrared (FTIR) spectroscopy: towards a high-throughput screening assay for antibiotic discovery and mechanism-of-action elucidation. Metabolites 10:145
14. Ducournau A, Bénéjat L, Sifré E, Bessède E, Lehours P, Mégraud F (2016) Helicobacter pylori resistance to antibiotics in 2014 in France detected by phenotypic and genotypic methods. Clin Microbiol Infect 22:715–718
15. Zeng R, Tao J, Tang D, Knopp D, Shu J, Cao X (2020) Biometric-based tactile chemomechanical transduction: an adaptable strategy for portable bioassay. Nano Energy 71:104580
16. Zeng R, Su L, Luo Z, Zhang L, Lu M, Tang D (2018) Ultrasensitive and label-free electrochemical aptasensor of kanamycin coupling with hybridization chain reaction and strand-displacement amplification. Anal Chim Acta 1038:21–28
17. Zeng R, Zhang L, Luo Z, Tang D (2019) Palindromic fragment-mediated single-chain amplification: an innovative mode for photoelectrochemical bioassay. Anal Chem 91:7835–7841
18. Zeng R, Luo Z, Su L, Zhang L, Tang D, Niessner R, Knopp D (2019) Palindromic molecular beacon based Z-scheme BiOCl-Au-CdS photoelectrochemical biodetection. Anal Chem 91:2447–2454
19. Zeng R, Tang Y, Zhang L, Luo Z, Tang D (2018) Dual-readout aptasensing of antibiotic residues based on gold nanocluster-functionalized MnO_2 nanosheets with target-induced etching reaction. J Mater Chem B 6:8071–8077
20. Maity S, Bain D, Patra A (2019) An overview on the current understanding of the photophysical properties of metal nanoclusters and their potential applications. Nanoscale 11:22685–22723
21. Jain U, Gupta S, Chauhan N (2019) Construction of an amperometric glycated hemoglobin biosensor based on Au–Pt bimetallic nanoparticles and poly (indole-5-carboxylic acid) modified Au electrode. Int J Biol Macromol 105:549–555
22. Blanco-López MC, Rivas M (2019) Nanoparticles for bioanalysis. Anal Bioanal Chem 411:1789–1790
23. Weng X, Cai W, Lan R, Sun Q, Chen Z (2018) Simultaneous removal of amoxicillin, ampicillin and penicillin by clay supported Fe/Ni bimetallic nanoparticles. Environ Pollut 236:562–569
24. Tabrizian P, Ma W, Bakr A, Rahaman MS (2019) pH-sensitive and magnetically separable Fe/Cu bimetallic nanoparticles supported by graphene oxide (GO) for high-efficiency removal of tetracyclines. J Colloid Interface Sci 534:549–562
25. Wu H, Feng Q, Lu P, Chen M, Yang H (2018) Degradation mechanisms of cefotaxime using biochar supported Co/Fe bimetallic nanoparticles. Environ Sci Water Res Technol 4:964–975
26. Zhang Y, Zhang BT, Teng Y, Zhao J, Kuang L, Sun X (2020) Carbon nanofibers supported Co/Ag bimetallic nanoparticles for heterogeneous activation of peroxymonosulfate and efficient oxidation of amoxicillin. J Hazard Mater 400:123290
27. Kang S, Xia F, Hu Z, Hu W, She Y, Wang L, Fu X, Lu W (2020) Platinum nanoparticles with TiO2–skin as a durable catalyst for photoelectrochemical methanol oxidation and electrochemical oxygen reduction reactions. Electrochim Acta 343:136119
28. Košević MG, Zarić MM, Stopić SR, Stevanović JS, Weirich TE, Friedrich BG, Panić VV (2020) Structural and electrochemical properties of nesting and core/shell Pt/TiO2 spherical particles synthesized by ultrasonic spray pyrolysis. Metals 10:11
29. Hanada N, Kohase Y, Hori K, Sugime H, Noda S (2020) Electrolysis of ammonia in aqueous solution by platinum nanoparticles supported on carbon nanotube film electrode. Electrochim Acta 341:136027
30. Chauhan N, Narang J, Jain U (2015) Highly sensitive and rapid detection of acetylcholine using an ITO plate modified with platinum-graphene nanoparticles. Analyst 140:1988–1994
31. Ozcelikay G, Kurbanoglu S, Yarman A, Scheller FW, Ozkan SA (2020) Au-Pt nanoparticles based molecularly imprinted nanosensor for electrochemical detection of the lipopeptide antibiotic drug daptomycin. Sensors Actuators B Chem 320:128285
32. Zeng R, Luo Z, Zhang L, Tang D (2018) Platinum nanozyme-catalyzed gas generation for pressure-based bioassay using polyaniline nanowires-functionalized graphene oxide framework. Anal Chem 90:12299–12306
33. Zeng R, Wang W, Cai G, Huang Z, Tao J, Tang D, Zhu C (2020) Single-atom platinum nanocatalyst-improved catalytic efficiency with enzyme-DNA supermolecular architectures. Nano Energy 74:104931
34. Yu Z, Cai G, Liu X, Tang D (2020) Platinum nanozyme-triggered pressure-based immunoassay using a three-dimensional polypyrrole foam-based flexible pressure sensor. ACS Appl Mater Interfaces 12:40133–40140
35. Aydoğdu G, Zeybek DK, Pekyardımcı Ş, Kılıç E (2013) A novel amperometric biosensor based on ZnO nanoparticles-modified carbon paste electrode for determination of glucose in human serum. Artif Cells Nanomed Biotechnol 41:332–338

36. Al-Mohaimeed AM, Al-Onazi WA, El-Tohamy MF (2020) Utility of zinc oxide nanoparticles catalytic activity in the electrochemical determination of minocycline hydrochloride. Polymers 12:2505
37. Narang J, Chauhan N, Pundir S, Pundir CS (2013) A magnetic nanoparticles-zinc oxide/zinc hexacyanoferrate hybrid film for amperometric determination of tyrosine. Bioprocess Biosyst Eng 36:1545–1554
38. Jasiecki S, Czupryniak J, Ossowski T, Schroeder G (2013) FTO coated glass electrode functionalization with transition metal cations receptors via electrostatic self-assembly. Int J Electrochem 8:12543–12556
39. Chauhan N, Tiwari S, Narayan T, Jain U (2019) Bienzymatic assembly formed@ Pt nano sensing framework detecting acetylcholine in aqueous phase. Appl Surf Sci 474:154–160
40. Ghorbani HR, Mehr FP, Pazoki H, Rahmani BM (2015) Synthesis of ZnO nanoparticles by precipitation method. Orient J Chem 31:1219–1221
41. Hatada M, Tsugawa W, Kamio E, Loew N, Klonoff DC, Sode K (2017) Development of a screen-printed carbon electrode based disposable enzyme sensor strip for the measurement of glycated albumin. Biosens Bioelectron 88:167–173
42. Lee CW, Su H, Cai YD, Wu MT, Wu DC, Shiea J (2017) Rapid identification of psychoactive drugs in drained gastric lavage fluid and whole blood specimens of drug overdose patients using ambient mass spectrometry. Mass Spectrom 6:S0056
43. Jain U, Gupta S, Chauhan N (2017) Detection of glycated hemoglobin with voltammetric sensing amplified by 3D-structured nanocomposites. Int J Biol Macromol 101:896–903