Fabrication of Electrochemical Biosensor Using Zinc Oxide Nanoflowers for the Detection of Uric Acid

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Abstract: A label-free electrochemical biosensor has been developed using zinc oxide nanoflowers (ZnONFs) for the detection of uric acid concentration in human blood serum. ZnONFs have been synthesized by a hydrothermal process and characterized with several techniques such as ultraviolet–visible (UV–Vis) spectroscopy, Fourier transform infrared spectroscopy (FTIR) studies, X-ray diffraction (XRD) study, Raman spectroscopy, scanning electron microscopy (SEM) and high-resolution transmission electron microscopy (HR-TEM) and electrochemical analyzer to confirm the formation of nanoflowers and fabrication of electrode and bio-electrodes for uric acid detection. Zinc Oxide nanoflowers have been deposited onto indium–tin oxide (ITO) substrate through electrophoretic deposition technique, and the biosensor has been fabricated by immobilizing urate oxidase (UOx) enzyme onto ZnONFs/ITO electrode surfaces. Further, electrochemical studies have been performed with immobilized UOx/ZnONFs/ITO bio-electrode as a function of uric acid concentrations. It has been found that the fabricated uric acid biosensor shows a high sensitivity (10.38 lA/mM/cm²) and a limit of detection of 0.13 mM in the range of 0.005 to 1.0 mM. This study demonstrates the potential use of ZnONFs for the construction of sensitive biosensors for uric acid detection.

Keywords: Uric acid; Uricase; Horseradish peroxidase (HRP); ZnONFs; Cyclic voltammetry; Urea; Electrochemical sensor

1. Introduction

Uric acid is an end product in the metabolic processes of purine nucleotides in the human body. Uric acid concentration in blood can be determined using various clinical methods such as spectrophotometric method using an enzyme–analyte reaction mechanism using uricase enzyme in the presence of 4-aminoantipyrine and 2,4,6-tribromophenol. The permissible concentration of uric acid in the human serum lies in the range 237.9–356.9 μmol/L (4 to 6 mg/dL) for males and 178.4–279.4 μmol/L (3 to 5 mg/dL) for females [1, 2]. The presence of optimum amount of uric acid in the human serum is required in maintaining the function of renal system. An increase in the concentration of uric acid beyond the allowed permissible limit can lead to severe chronic and acute diseases such as cardiovascular disease, hyperuricemia, hypouricemia, gout, arthritis, renal system problems and chronic kidney disease (CKD) [3]. Nanomaterials play an important role in improving the performance of biosensor due to high electrical, mechanical, optical, and catalytic properties. Further, for the improvement in the performance of biosensor, nanomaterials such metal oxide and nanoparticles have been explored recently, which lead to enhance the surface-to-volume ratio, biocompatibility, charge carrier mobility and mechanical flexibility. Biosensors are utilized for non-clinical as well as clinical laboratories using the specific bioreceptor bio-analyte system [2, 4]. Various uric acid biosensors have been reported utilizing metal oxide nanoparticles and uricase–horseradish peroxidase (HRP) (UOx) enzymes for fabrication of uric acid biosensors [5]. Chauhan et al. have developed a uric acid biosensor using the multiwalled carbon nanotubes as a transducer that is deposited onto Au electrode surface. The fabricated biosensor has a high sensitivity of 0.44 mA mM⁻¹ with the linear range of 0.01–0.8 mM and a limit of detection of 0.01 mg/mL. A high stability and a shelf life of the bio-
electrode surface demonstrate the effective immobilization of enzymes [6]. Muhsin Ali et al. have developed the Zinc Oxide quantum dots (ZnQDs)-based uric acid biosensors using uricase enzyme to fabricate the uric acid biosensor. This sensor possesses a high sensitivity of 4.0 µA/mM cm⁻² [7]. Shefali Jain et al. reported a uric acid biosensor with a sensitivity of 1.838 µA/(µM-cm²) and a LOD of 0.066 µM with the linear range of 0–700 µM by using butylamine-capped spherical CZTS nanoparticles deposited onto ITO with uricase enzyme [8]. Castillo-Ortega et al. have constructed polyaniline-poly-n-butyl-methacrylate composite films immobilized with uricase enzyme (urate oxidase) for the detection of uric acid [9]. Uric acid biosensors showed 3 months shelf life. Zhang et al. reported ZnO nano rods based uric acid biosensors and the shelf life of constructed bio-electrode is around 20 days [10, 11]. Kan et al. developed a polyaniline-urate case bio-electrode-based biosensor via methods of the generation of hydrogen peroxide and its utilization by peroxidase that detect the decreasing level of dissolved oxygen concentration by the constructed biosensor. Liu et al. have constructed a bio-electrode in which a self-assembled monolayer contains a novel norbornylogous bridge by covalently attaching to flavin adenine dinucleotide (FAD), the redox active center of several oxidase enzymes [12, 13].

M. M. Alam et al. developed a UA biosensor based on ZnO/Ag2O/Co3O4NPs/binder/GCE with the linear range of 0.1 nM–0.01 mM and showed a selective study of uric acid with uricase enzyme [14]. UOX enzymes used for the detection of uric acid help to catalyze the oxidation reaction of uric acid that is breakdown into allantoin and H₂O₂ to produce electrons for sensitivity [15]. The fabricated uric acid detection is mediator-free [16]. This uric acid biosensor is based on electrochemical reactions occurring onto working electrodes. An electrochemical signal is produced by an electron conductivity present in the buffer solution, and the electrochemical reactions are processed [17–24]. Zinc Oxide nanostructures have been reported to enhance the electrochemical and electrocatalytic properties after deposition onto the ITO/GCE electrodes as they enhance an electroactive surface area [25, 26]. As morphology plays a very important role in enhancing the conductivity and electrochemical behavior, it further may enhance the sensitivity of biosensor. In the present work, efforts have been made to fabricate a uric acid biosensor using the nanostructured zinc oxide matrix having flower-like morphology. ZnO is a highly remarkable material because of its specific properties such as wide band gap (3.32 eV), semiconductor in nature and high isoelectric point (IEP) 9.5. ZnO exhibits a high IEP value which directly gets attached with a low IEP value of uricase enzyme (4.5), which results in strong physical bonding formation taking place between nanomaterial and enzyme that enhances the stability of the biosensor. Hydrothermal processes have been utilized to synthesize nanoflower that is deposited onto an indium–tin oxide (ITO) glass electrode surface using an electrophoretic deposition technique (EPD) under the optimized applied voltage. In the present study, the fabrication of uric acid biosensor using the nanostructured Zinc Oxide has been carried out and the performance has been compared with that of the reported literature.

2. Experiments

2.1. Reagents and Materials

Uricase (urate oxidase, EC 1.7.3.3 from Bacillus fastidiosus, 9U/mg), horseradish peroxide (HRP) and uric acid (2,6,8-hydroxypurine, monosodium salt) were purchased from Sigma Chemical Co., St. Louis, MO, USA. Zinc acetate (C₃H₆O₂Zn), sodium hydroxide (NaOH) and all utilized chemicals were purchased from Sigma-Aldrich and used without further purification. Potassium ferrocyanide (K₄[Fe(CN)₆]), potassium ferricyanide (K₃[Fe(CN)₆]), sodium dihydrogen phosphate (NaH₂PO₄), sodium mono-hydrogen phosphate (Na₂HPO₄), aqueous solutions and buffers were prepared in Milli-Q water (18 MΩcm), urea, cholesterol, ascorbic acid and glucose.

2.1.1. Instruments

The ZnONFs were characterized using various techniques such as UV–visible spectrophotometer (lambda 950, PerkinElmer), Fourier transform infrared (FTIR) spectroscopy (Spectrum BX, PerkinElmer) and X-ray diffractometer (Cu Kα radiation, Rigaku, Japan). Morphological investigations of ZnONFs were studied using a scanning electron microscope (SEM) (SEM, LEO 440) and a high-resolution transmission electron microscope (HR-TEM, Tecnai-G2F30 STWIN). Sensing measurements were recorded using an Auto lab/galvanostat/potentiostat (model AUT 84.275, Eco Chemie, The Netherlands) in phosphate-buffered saline (PBS, 50 mM, 0.9% NaCl, pH 7.4) containing 5 mM [Fe(CN)₆]³⁻/⁴⁻ (redox species) with a three-electrode system in which Ag/AgCl is used as the reference electrode and platinum (Pt) as the counter electrode at about 25 °C. A pH meter was calibrated with a reference material provided by Bharatiya Nirdeshaka Dravya (BND) at CSIR-NPL. Weighing balance was calibrated using standard weights provided by manufacturer, FTIR was calibrated with NIST-supplied polystyrene films reference standard. All the instruments were self-calibrated before use as per manufacturer’s guidelines.
2.1.2. Synthesis of Zinc Oxide Nanoflowers (ZnONFs)

A hydrothermal process is a promising method to synthesize nanostructures with a controlled shape and size. Here, ZnONFs were synthesized using a low-temperature method. In brief, a solution of 0.1 M concentration of zinc acetate (C_4H_6O_4Zn) and base sodium hydroxide (NaOH) was prepared in 50 mL of deionized water. Thereafter, this solution was transferred into a Teflon seal tube and the tube was placed in a furnace at 80 °C for 18 h. Thereafter, the tube was kept at room temperature to let it cool down and the synthesized material was washed several times with ethanol and deionized water at 3000 rpm. After separating precipitate, it was further incubated in a preheated incubator at 60 °C for 24 h to obtain Zinc Oxide powder.

2.1.3. Electrophoretic Deposition of ZnO Nanostructures

Before the deposition of ZnONFs material over ITO substrate, first, ITO glass electrodes (size: 1 cm × 2 cm) were hydrolyzed in solution containing 5:1:1 v/v of H_2O/H_2O_2/9M glacial acetic acid (C_4H_6O_4Zn) and base sodium hydroxide (NaOH) was prepared in 50 mL of deionized water. Thereafter, this solution was transferred into a Teflon seal tube and the tube was placed in a furnace at 80 °C for 18 h. Thereafter, the tube was kept at room temperature to let it cool down and the synthesized material was washed several times with ethanol and deionized water at 3000 rpm. After separating precipitate, it was further incubated in a preheated incubator at 60 °C for 24 h to obtain Zinc Oxide powder.

2.1.4. Fabrication of UOx/ZnONFs/ITO Bio-Electrode by Physical Adsorption

For the fabrication of biosensor, the selection of the material, the uniform deposition of the film, immobilization and a high coverage of enzyme plays a crucial role. The UOx/ZnONFs/ITO bio-electrode was constructed by a physical adsorption process. Firstly, the stock solution of uricase enzyme (1 mM) was prepared using a solution of Tris–HCl buffer (1 M) and HRP (0.5 mM). Further, uricase enzyme (0.16U/18µL) and 10 µL of HRP were drop-casted onto ZnONFs/ITO electrodes. Then, they were placed in a humid chamber for 3 h and then dried at room temperature. Finally, the prepared UOx/ZnONFs/ITO bio-electrodes were stored in the refrigerator at 4 °C, when not in use.

3. Results and Discussion

3.1. UV–Visible Spectroscopy Study

An UV–visible spectrophotometer was utilized in the range of 250 to 850 nm using a quartz cuvette for the optical study of synthesized ZnONFs. An UV–visible absorption peak was observed at 370 nm for ZnONFs material [Fig. 2(a)]. This absorption peak indicates the presence of n-IT* antibonding due to the presence of lone-pair electrons of oxygen atom. This shift corresponds to the structure of ZnONFs. The energy band gap was calculated and found to be 3.35 eV [27].

3.2. Fourier Transform Infrared Spectroscopy Study

The presence of functional groups in the ZnONFs structure was confirmed by a FTIR spectrophotometer. The characteristic peak of zinc oxide was observed in the fingerprint region of 400–550 cm\(^{-1}\) [Fig. 2(b)]. The characteristic peak at 3500 cm\(^{-1}\) was observed due to the hydroxyl groups. However, after uricase immobilization onto the ZnO NF/ITO electrodes, the additional peaks observed at 1410 cm\(^{-1}\) and 1575 cm\(^{-1}\) are attributed to the C = O stretching vibrations and the peak observed at 3050 cm\(^{-1}\) corresponds to the O–H stretching vibrations [28]. The peak observed at 2350 cm\(^{-1}\) is attributed to the C = O stretching vibrations [29, 30]. Other peaks observed at 1558 cm\(^{-1}\) and 1649 cm\(^{-1}\) correspond to the primary and secondary linkage of amide groups present in the UOx enzyme.

3.3. X-Ray Diffraction Pattern Analysis

A typical X-ray diffractometer source of copper target of 30 kV and 15 mA was used with a scan rate of 3/min. X-Ray diffraction patterns depicted in Fig. 2 (c) have 20 (20 to 80). Fine and sharp peaks were observed at nine different positions in XRD crystallographic patterns corresponding to (100), (002), (101), (102), (110), (103), (200), (112) and (201) crystal faces. The observed peaks in these data were matched with the certified XRD pattern of ZnO with JCPDS card no. 89–1397. This JCPDS card information revealed that the synthesized ZnONFs are in a hexagonal wurtzite form with crystalline nature. The crystallite size of ZnONFs was calculated to be 17 nm. No peaks were observed from impurities, which indicates that the product is pure [31].

3.4. Raman Studies

A Raman spectrum was recorded in the range from 100 cm\(^{-1}\) to 1000 cm\(^{-1}\) Raman shift. The Raman
The spectrum of ZnONFs is depicted in Fig. 2 (d), revealing the formation of crystallographic ZnONFs. The Raman scattering reveals that ZnONFs have a hexagonal structure with $C_{6v}$ space group. A symmetry of ZnONFs structure shows that vibrations are Raman active in other possibilities for other factors such as lattice spacing, and the chemical environment determines the place of vibration frequencies. A sharp and strong peak at 438 cm$^{-1}$ of optical photon $E_{2h}$ mode reveals that this structure has a good crystalline structure [32]. A peak at 99 cm$^{-1}$ corresponding to $E_{2L}$ low-intensity mode indicates this structure has some crystallite defects also [33].

3.5. Morphological Studies

Morphological studies of ZnONFs were carried out using SEM technique, and the results are depicted in Fig. 3a and 3b. Figure 3a indicates the SEM image highlighting at 1 µm scale bar reveals the well-dispersed and uniform nanomaterial deposition onto ITO-coated glass substrates. Figure 3b indicates the SEM image at higher scale bar reveals the flower-like morphology of nanoparticles [34]. The high-resolution transmission electron microscope (HR-TEM) images and their fringes were recorded and are shown in Fig. 4a and 4b. Figure 4a indicates the formation of flower-like morphology of ZnO nanostructure deposition, and Fig. 4b demonstrates the information of inter-planar distances by fringes image, structure, shape and size of the synthesized ZnONFs. These figures indicate that an irregular structure of ZnONFs consisting of simple needle flowers has inter-planar spacing (d) about 0.28 nm for (100) planes. These results revealed that ZnO exhibits a hexagonal structure with a size of 2.6 nm. The inset image in Fig. 4(b) is attributed to the synthesized nanoflowers structures in a well crystalline form and in fine structures. The SEAD pattern (ring-like shape) confirms ZnONFs synthesized in a polycrystalline form.

3.6. Electrochemical Studies

The electrochemical studies of the fabricated electrode and bio-electrode were performed using the cyclic voltammetry technique in a three-electrode system of Ag/AgCl as a reference electrode and platinum as a counter electrode and UOx/ZnONFs/ITO bio-electrode as a working electrode in PBS, pH 7.4 (50 mM, 0.9% NaCl), containing 5 mM [Fe(CN)$_6$]$^{3-}$/$4-$ at about 25 °C at a scan rate ($v$) of 50 mV/s. The cyclic voltammograms of the ITO electrodes were measured before and after the surface modification with ZnONFs and UOx subsequently as shown in Fig. 5(a). It has been found that a bare ITO electrode shows oxidation peak current ($I_{pa}$) and reduction peak current ($I_{pc}$) values of 490 µA and -403 µA, respectively. The increase in $I_{pa}$ and $I_{pc}$ values was found after the deposition of ZnONFs onto ITO electrodes as 736 µA and -616.8 µA, respectively. The UOx enzyme was used for the oxidation of uric acid.
Fig. 2  a UV–visible spectra of ZnONFs, b FTIR spectra of ZnONFs pellet and of UOx/ZnONFs/ITO bio-electrodes, c X-ray diffraction pattern of ZnONFs powder, d Raman spectra of ZnONFs powder

Fig. 3  Scanning electron microscopy (SEM) images of ZnONFs/ITO electrode
Fig. 4 Transmission electron microscopy (TEM) images of ZnONFs drop-casted onto copper TEM grid

Fig. 5  

(a) Cyclic voltammetry response of (i) ITO, (ii) ZnONFs/ITO electrode and (iii) UOx/ZnONFs/ITO bio-electrodes conducted in 50 mM PBS (pH 7.4) containing 5 mM \([\text{Fe (CN)}_6]^{3+/4-}\) redox species at a scan rate of 50 mV s\(^{-1}\).  
(b) Cyclic voltammetry studies as a function of scan rate from 10 to 100 mV s\(^{-1}\) for UOx/ZnONFs/ITO bio-electrode conducted in 50 mM PBS (pH 7.4) containing 5 mM \([\text{Fe (CN)}_6]^{3+/4-}\) redox species. Inset image: anodic peak current and cathodic peak current vs. square root of the scan rate.  
(c) Variation in current (Ip) of oxidation peaks with scan rate (v) and  
(d) variation of anodic current(Ip) in log with log scan rate (v)
(C₅H₄N₄O₃) into allantoin (C₄H₆N₄O₃) and HRP which helps in catalysis process of H₂O₂ into 2H⁺ + O₂ + 2e⁻.

The reactions can be described in Eqs. (1) and (2):

\[
\text{H₂O₂} \xrightarrow{\text{HRP}} 2\text{H}^+ + \text{O}_2 + 2\text{e}^- \quad (2)
\]

After the immobilization of enzymes onto the ZnONFs/ITO electrode, Iₚa and Iₚc values were found to be decreased as 620µA and -507.5µA, respectively. This may be attributed to the immobilization of insulating nature of the enzyme UOx onto ZnONFs/ITO electrodes.

3.7. Redox Reaction Occurs in PBS Onto UOx/ZnONFs/ITO Bio-Electrodes

A cyclic voltammetry response of UOx/ZnONFs/ITO bio-electrodes was obtained at scan rates from 10 to 100 mV/s, and the recorded curve is shown in Fig. 5(b). The CV curves revealed that Iₚa and Iₚc increased in both directions simultaneously by varying the scan rate values. A peak current ratio (Iₚa/Iₚc) of UOx/ZnONFs/ITO bio-electrode was found to be 1, which indicates the quasi-reversible behavior of redox species. The inset image in Fig. 5(b) shows the redox current response of UOx/ZnONFs/ITO bio-electrode plotted with respect to the square root of scan rate. An anodic and cathodic peak current can be established with the help of Eqs. 3 and 4, respectively [35]:

\[
I_{pa} = -35.69 \mu A + 67.44 \\
\times 10^{-6} (A^2\text{mV s}^{-1})^{1/2} \text{[scan rate (mV s)}^{-1}]^{1/2}; R^2 = 0.9995
\]

\[
I_{pc} = -16.13 \mu A - 54.60 \\
\times 10^{-6} (A^2\text{mV s}^{-1})^{1/2} \text{[scan rate (mV s)}^{-1}]^{1/2}; R^2 = 0.9998
\]

In the Randles–Sevcik equation, the current peak intensity mainly depends upon two factors: The first is the working electrode active surface area and the second is the concentration of electroactive species which effectively contributes to the performance of biosensor. The value of diffusion coefficient (D) can be calculated using the Randles–Sevcik equation (Equation no. 5):

\[
I_{pa} = 2.69 \times 10^5 n^{3/2} AD^{1/2} u^{1/2} C.
\]

In this equation, Iₚa stands for the peak current, n stands for the number of electrons, C stands for the concentration of redox species, D stands for the diffusion coefficient, A stands for the electroactive surface area and v stands for the scan rate. The calculated diffusion coefficient (D) values for ITO electrode, ZnONFs/ITO electrode and UOx/ZnONFs/ITO electrode are found to be 2.438 × 10⁻⁶ cm² s⁻¹, 5.5 × 10⁻⁶ cm² s⁻¹ and 3.949 × 10⁻⁶ cm² s⁻¹, respectively. The high value of diffusion coefficient for ZnONFs/ITO electrode in comparison with ITO electrode is attributed to the presence of nanomaterial deposited onto ITO electrode in the case of ZnONFs/ITO, and this coating of nanomaterial improved the electron activity, surface-to-volume ratio and kinetics of the reaction performed in the redox solution with the variation concentration of analyte. But, after immobilization of UOx enzyme onto ZnONFs/ITO electrode, the diffusion coefficient value 3.949 × 10⁻⁶ cm² s⁻¹ was found to be slightly lower as compared to ZnONFs/ITO electrode. It is because UOx enzyme reduces the electron activity of material onto the bio-electrode surface because it behaves as a non-conducting layer and promotes the hindrance in the electron transfer process. These heavy molecules restrict the electron interaction and create hindrance in electron transportation [36].

A current response (oxidation) Iₚ in Fig. 5(c) has linear dependence on a scan rate v¹/₂, signifying that the electrochemical reaction is diffusion controlled [37]. This linear dependency can be explained by the following equation:

\[
I_{pa} \propto v^{1/2}.
\]
\[ I_p = 68.0253 + 39.347 \, v^{1/2}; \, R^2 = 0.9995. \]  

Equation (6)

Figure 5(d) shows the scan rate and current response (Ip) in the form of log linear plot, which is explained in the following equation:

\[ \log I_p = 1.6850 + 0.5631 \, \log v^{1/2}; \, R^2 = 0.9967. \]  

Equation (7)

3.8. Electrochemical Biosensing Response of bio-Electrode for Uric Acid Detection

3.8.1. Metrological Aspects of Uric Acid Detection

All the sensing study was carried out at a controlled temperature of 23 ± 2 °C and a humidity of 50 ± 10% RH. The spiked solution of uric acid was prepared using a calibrated microbalance and micropipettes. The response study data used for the calibration of bio-electrode for uric acid detection were taken from three repeated experiments (n = 3), and type-A uncertainty was calculated. The uncertainty from other sources such as mass balance and micropipettes traceable to national standard was included during sample preparation itself. The calibration curve of the electrochemical response of the bio-electrode was plotted as a function of uric acid concentration in PBS with the incorporation of type-A uncertainty (Table 1) as shown in Fig. 6b.

3.8.2. Electrochemical Response of Bio-Electrode

The biosensing response of the fabricated bio-electrodes was recorded as a function of uric acid concentrations in the range of 0.005 to 0.75 mM, and the response characteristics are depicted in Fig. 6(a). It has been found that the current response is increased with an increase in the concentration of uric acid indicating the binding between enzyme and uric acid due to the increasing rate of H₂O₂ molecule, which increases the transfer of number of electrons during the reaction process. The calibration curve was plotted and is depicted in Fig. 6(b), which shows that a linear increment in the current value with increasing the concentration of uric acid in the range of 0.005–0.75 mM in PBS indicates a linear behavior of the biosensor.

\[ I_p = 732.5 \, \mu A + 10.55 \, \mu A/\text{mM/cm}^2 \times \text{concentration (mM)}; \, R^2 = 0.958 \]  

Equation (8)

LOD = 3σ/m  

Equation (9)

where σ is the standard deviation of blank solutions and m is the slope of the calibration curve.

The fabricated UOx/ZnONFs/ITO bio-electrode shows a sensitivity of 10.38 μA/ mM/cm², a theoretical LOD value of 0.13 mM and an experimental value of 0.005 mM, which can be calculated by using Eq. no. 10. The value of sensitivity and LOD of UOx/ZnONFs/ITO bio-electrode was found in exceedingly high range because of some specific reasons such as deposition of ZnONFs onto ITO electrodes that has improved the electron conductivity, electron affinity, electroactivity and surface-to-volume ratio of ZnONFs/ITO electrodes. In the case of Ferri–Ferro buffer solution, Fe³⁺ is reduced via heterogeneous electron transfer from the fabricated bio-electrode. This fabricated UOx/ZnONFs/ITO bio-electrode behaves as an electrical conductor and Pt electrode behaves as a potentiated (which can control the energy of the electron initiated from bio-electrode). Due to the enhancement of electron conductivity from UOx/ZnONFs/ITO bio-electrode, the peak current has a slightly higher range in comparison with ITO electrodes. An enzyme immobilization process also plays a major role in the detection of uric acid. UOx enzyme is immobilized onto a material electrode surface followed by a drop-casting process. Basically, UOx enzymes help to oxidize uric acid into allantoin, CO₂ and H₂O₂. In the second process, H₂O₂ releases electrons, and these electrons initiate conductivity and give peak current after applying voltage. The electron conductivity increased with the concentration of uric acid in buffer solution.

| Matrix                              | Sensitivity          | Detection range   | LOD       | References |
|-------------------------------------|----------------------|-------------------|-----------|------------|
| Uricase/AuNPs/MWCNTs/Au             | 0.44 mA mM⁻¹ cm⁻²    | 0.01–0.8 mM       | 0.01 mM   | [6]        |
| UOx/Fe/Cu₂O/GCE                     | 1.900 μA · mM⁻¹ cm⁻²| 0.01–1 mM         | 0.0596 μM | [38]       |
| Uricase/ZnONRs                      | 0.1054 μA/mM         | 5 μM-3 mM         | –         | [11]       |
| Naftion/ZnOQDs/Uricase              | 4.0 μA/mM.cm⁻²       | 1 mM-10 mM        | 22.97 μM  | [7]        |
| Butylamine-capped spherical CZTS nanoparticles | 1.838 μA/μM.cm² | 0–700 μM          | 0.066 μM  | [8]        |
| CNF-RGO                             | 0.14 μA/μM           | 100–700 μM        | –         | [39]       |
| Graphene/Pt-GCE                     | 0.41 μA/μM           | 0.05–11.85 μM     | –         | [40]       |
| Bacillus uricase/ PANI/MWCNT/ITO electrode | –                 | 0.005–0.6 mM      | 0.005 mM  | [1]        |
| UOx/ZnONFs/ITO bio-electrode        | 10.38 μA/mM/cm²      | 0.005–0.75 mM     | 0.13 ± 0.1 mM | This work |

Table 1 Comparison of several fabricated biosensors and their activity
3.8.3. Interference and Stability Studies

The uric acid biosensor helps to regulate the concentration of uric acid in the human blood serum, but in the human blood serum there are several other analytes present. Some of these analytes can interfere in this specific detection of uric acid. To check the specificity, we have studied the performance of the biosensor by mixing 1 mM of uric acid with different potential interferents such as urea (2 mM), ascorbic acid (3.8 mM), glucose (5 mM) and cholesterol (3.8 mM) [Fig. 7].

\[
\text{interference} = \left( \frac{I_{FD} - I_{Int}}{I_{FD}} \right) \times 100
\]

(10)

By using Eq. no. 11, the calculated value of interference with analyte was found to be exceptionally low (less than 1), so this value is completely negligible. This proves that UOx/ZnONFs/ITO bio-electrode is specific and selective for the detection of uric acid in the human blood serum. This proves that UOx/ZnONFs/ITO biosensor can detect only uric acid in the human blood serum apart from the...
other analytes present in serum such as glucose and cholesterol. The studies on shelf life of the fabricated biosensor were carried out using the bio-electrodes for uric acid detection, and the response studies were carried out after storing them for 5 weeks at 4 °C. The response characteristics have indicated that above 85% of the retention in the current was observed, indicating that the electrodes can be stored for 5 weeks at 4 °C.

Table 1 represents the performance of the fabricated uric acid biosensors in comparison with the earlier reported uric acid biosensors. The detection range and detection limit of UOx/ZnONFs/ITO bio-electrode as reported in this paper are wider and higher than those of the other reported uric acid biosensors.

4. Conclusions

In summary, we have fabricated a sensitive and selective uric acid biosensor ZnONFs. The ZnONFs help to detect a low concentration of uric acid because of their improved surface-to-volume ratio, enhanced electrocatalytic properties and improved electrical redox behavior. The UOx/ZnONFs/ITO bio-electrode-based biosensor helps to detect the uric acid concentration from 0.005 to 1.0 mM, exhibiting LOD (0.13 mM) in a redox probe. This biosensor shows a high selectivity and specificity with a high sensitivity of 10.38 µA/Mm/cm². We could not get real samples from the clinical laboratories due to COVID pandemic restrictions. We will take up this work in future to validate the biosensor with real samples in near future.

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Authors contribution All authors have contributed equally to this work.

Declaration

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies related to human participants or animal performed.

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