I, Ravindra Singh, hereby wholly retract this Analyst paper. Signed: R Singh, India, June 2011. This retraction is endorsed by May Copsey, Editor. Retraction published July 2011.

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PAPER

A catechol biosensor based on a gold nanoparticles encapsulated-dendrimer

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Tyrosinase has been immobilized on a Au nanoparticles encapsulated-dendrimer bonded conducting polymer on a glassy carbon electrode for the estimation of catechol. The modified electrode was characterized by cyclic voltammetry and AFM techniques. The principle of catechol estimation was based on the reduction of biocatalytically liberated quinone species at +0.2 V *versus* Ag/AgCl (3 M KCl), with good stability, sensitivity, and featuring a low detection limit (about 0.002 μM) and wide linear range (0.005 μM–120 μM). The electrochemical redox peak of catechol on the GCE/PolyPATT/Den(AuNPs)/tyrosinase was also investigated. A response time of 7 s, reusability up to 5 cycles and a shelf life of more than 2 months under refrigerated conditions were reported. Various parameters influencing biosensor performance have been optimized including pH, temperature, and applied potential. The utility and application of this nanobiosensor was tested in a real water samples.

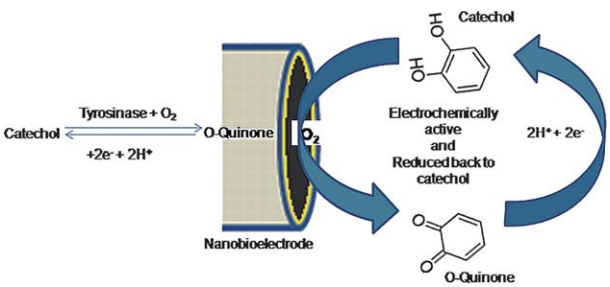
1. Introduction

Now, it has been established that the performance of biosensor depends on the influence imposed on biomolecules by immobilization, and the use of nanomaterials for the construction of biosensing devices, offer one of the most exciting approaches.[1] Catechol is a widely studied phenol that is a common byproduct of factory waste. It loses its hydrogen atoms from the hydroxyl groups and becomes an *o*-quinone. However, the catechol oxidation product *o*-quinone may interact with vital cellular components such as lipids, proteins and DNA and cause damage to the cells.[2,3] Catechol is a phenolic compound, causing detrimental health effects.[4] The estimation of catechol is costly and time-consuming, using sophisticated equipment, for example colorimetric detection,[5,6] optical detection,[7] and HPLC.[8] Among the many analytical methods for the measuring of phenolic compounds, electrochemical biosensors based on immobilized tyrosinase have received the most attention.[9,10] Tyrosinase catalyses the conversion of the phenolic substrate to the corresponding quinone species that can be electrochemically reduced and allows convenient low-potential detection of the phenolic analyte.[11] A variety of methods for the immobilization of tyrosinase with an electrochemical transducer have been reported including adsorption,[12] cross-linking,[13] carbon paste matrix, graphite epoxy composite electrodes,[14] polyaniline–polyphenol oxidase,[15] hydrogels,[16] electropolymerization,[17] self-assembled monolayers,[18] self-wiring,[19] silica sol–gel,[20] alumina sol–gel,[21] polyaniline–ionic liquid–carbon nanofiber composite[22] and ZnO nanoparticles[23] for the detection of phenolic compounds.

It may be noted in the present manuscript that tyrosinase immobilized dendrimer-encapsulated AuNPs on a modified GCE have been fabricated as bioelectrode for the estimation of catechol. The PAMAM dendrimer encapsulated AuNP was covalently immobilized to the Poly-PATT conducting layer through a covalent bonding between the carboxylic acid-terminated groups of PAMAM dendrimer and the amine groups of Poly-PATT. Tyrosinase was then covalently immobilized to carboxylic acid terminated groups of PAMAM dendrimers. The Poly-PATT/Den(AuNPs)/tyrosinase-modified electrode was used for the detection of a catechol at submicromolar concentrations. Various experimental parameters affecting the catechol detection were optimized. In addition, the biosensor was applied to real time samples for the detection of catechol. Scheme 1 shows the schematic representation of the catechol biosensor.

2. Experimental

Tyrosinase, catechol, hydrogen tetrachloroaurate, dichloromethane (99.8%, anhydrous, sealed under N_2 gas),



Scheme 1 Schematic representation of the catechol biosensor.

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1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and *N*-hydroxysuccinimide (NHS), were purchased from Sigma Co (USA). Tetrabutylammonium perchlorate (TBAP) was procured from Fluka, and purified according to the general method, followed by drying under under vacuum at 10^{-5} Torr. A third generation (*G* 3) poly(amidoamine) dendrimer (diameter, 3.6 nm) with surface terminated succinamic acid groups [PAMAM ($NHCOCH_2CH_2COOH$) 32] and sodium borohydride were purchased from Aldrich Co. PBS was prepared by modifying 0.1 M disodium hydrogen phosphate (Aldrich) with the admixture of 0.1 M sodium dihydrogen phosphate (Aldrich) with 0.9% sodium chloride. Citrate buffer solution was prepared with 0.1 M citric acid and 0.1 M sodium citrate. Catechol stock solutions were prepared in a methanol–water mixture (1 : 4) and water respectively.

A glassy carbon electrode (area, 7 mm^2), Ag/AgCl (3 M KCl), and Pt wire were used as working, reference, and counter electrodes, respectively. Cyclic voltammograms (CVs) and amperograms were recorded using a potentiostat/galvanostat, Kosentech model KST-P2. Atomic force microscopy (AFM) was carried out on a multimode AFM system of Digital Instrument Inc. (USA) in tapping mode.

A functionalized conducting polymer layer with amine and immino groups (4′-pyrazine-2,2′,5′2″-terthiophene (PPATT)) was used in this study. A terthiophene monomer bearing a pyrazine amine group (PATT), was synthesized according to Kitamura *et al.*[24] A Poly-PATT film was grown on glassy carbon electrodes (GCE) through electropolymerization of the PATT monomer in a 0.1 M TBAP/CH_2Cl_2 solution by cycling the potential three times. After electropolymerization, the Poly-PATT film-coated GCE was washed with CH_2Cl_2 to remove any remaining monomers from the electrode surface. Prior to electropolymerization, GCEs were polished with 0.05 μm alumina/water slurry on a polishing cloth to a mirror finish, followed by sonicating and rinsing with distilled water. A 5.0 μM PAMAM (G3-COOH32) dendrimer solution was mixed with a 10 mM EDC solution by stirring for 6 h to activate surface carboxylic acid groups of the dendrimers. The Poly-PATT film-coated electrode was incubated for 6 h in the EDC treated dendrimer solution to covalently attach the dendrimer to the Poly-PATT film. The dendrimer attached Poly-PATT-coated electrode was then immersed in a 0.1 M $HAuCl_4$ solution with stirring for 1 h at room temperature. By this step, Au(III) ions were coordinated to nitrogen ligands in the interior of the dendrimer, which was covalently attached to the Poly-PATT film. A Den-AuNPs attached Poly-PATT (Poly-PATT/Den(AuNPs) electrode was then obtained by reducing Au(III) ions in the interior of the dendrimer with a 1.0 M $NaBH_4$ solution for 20 min. The Au nanoparticles encapsulated-dendrimer bonded Poly-PATT film-modified electrode was washed with distilled water and subsequently incubated for 12 h in a 0.1 M citrate buffer solution containing 23 U mL^{-1} tyrosinase. Different percentages of glutaraldehyde were used in the biosensor preparation for the determination of the effect of the glutaraldehyde on the biosensor response. For this purpose first three biosensors containing optimized tyrosinase (*i.e.* 23 U mL^{-1}) were treated with 2.50%, 5.0% and 10% glutaraldehyde solutions for covalent cross-linking. Only 5.0% concentration of glutaraldehyde in the biosensor preparation showed a linearity to detect catechol. So, the most useful and best responses were obtained by using the biosensor prepared with 5.0% glutaraldehyde. When considering this finding it is clear that an increase in the percentage of glutaraldehyde from 5.0% to 10.0% brought about an explicit decrease in biosensor response. The most important reason for this effect was the formation of more stringent cross-links. Hence catechol, the substrate molecule, could not be approached easily and converted into the product. The modified electrode was washed with distilled water two times to remove unbound tyrosinase from the dendrimer surface. The schematic representation of the modified electrode is shown in Fig. 1.

3. Results and discussion

The formation of the Poly-PATT film on a GCE was obtained through electropolymerization of a PATT monomer in a 0.1 M TBAP/CH_2Cl_2 solution using a potential cycling method using 0–1.4 V (*vs.* Ag/AgCl), scan rate 100 mV s^{-1} (Fig. 2). The CV recorded during polymerization exhibited oxidation peaks at +1.3 V *versus* Ag/AgCl during the first anodic scan, this was due to the oxidation of the monomer to form the polymer. The peak currents decreased as the cycle numbers increased, clearly demonstrating that the polymer film immediately formed after the oxidation of the PATT monomer at +1.3 V. A small and broad reduction peak of the polymer was observed at +0.9 V *versus* Ag/AgCl during the cathodic scan. The surface morphology of the conducting polymer film was characterized by AFM in order to investigate the homogeneity of the film. The AFM image of conducting polymer modified glassy carbon electrode is shown in Fig. 2 (inset). From the AFM image it can be concluded that the surface of the film has rough morphology and the polymer film is aggregated in round-shaped particles with high porosity, suggesting a high degree of polymerization. A single cycle grown polymer layer was used throughout the experiments for the preparation of the bioelectrode. The cyclic voltammogram was recorded for poly-PATT in a blank solution and redox peaks were found. Fig. 3 shows CVs at various scan rates suggesting that the peak current was directly proportional to the scan rate, and reveals the involvement of the surface adsorbed species. This finding suggests that the thickness of the film is thinner than the diffusion layer thickness of counter anions on the cyclic voltammetric time scale, through which the anions must diffuse in and out during the doping and dedoping processes.

The dendrimer and AuNPs were used to improve the performance of the tyrosinase immobilized electrode and act as a nanobioelectrode for the detection of nanomolar concentrations of catechol. Dendrimer is an insulator, it acts as not only a membrane but also provides enhanced stability and strong support to check damage of the conducting polymer and also strong attachment to AuNPs (intrinsically) for catalysis as well as more coverage area for enzyme attachment. In addition, gold nanoparticles play a significant role in the biosensor allowing communication between enzyme and electrode materials. For these reasons, it is expected that the utility of AuNPs can lead to enhancement of the performance of the tyrosinase electrode for the detection of catechol at low concentrations. Catechol was oxidized by tyrosinase as well as the electrochemical reaction

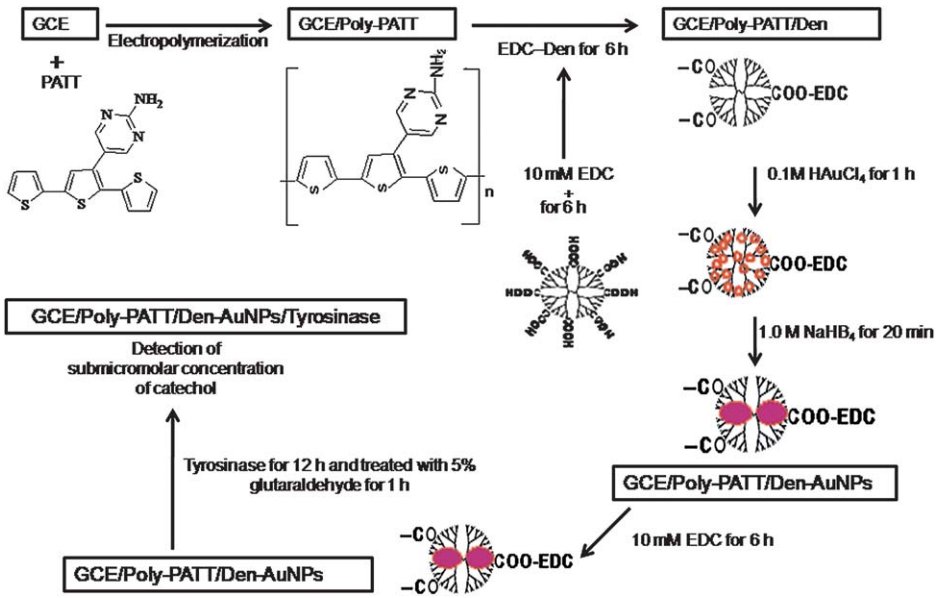

Fig. 1 The proposed schematic presentation of the nanobioelectrode.

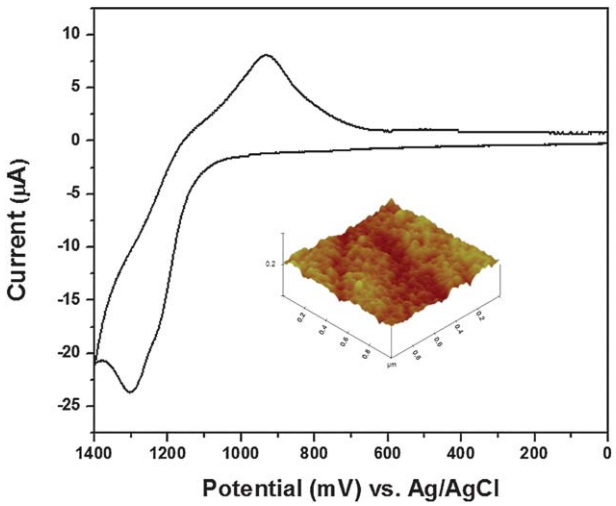

Fig. 2 CV for the electropolymerization of PATT for single cycle layer for bioelectrode preparation and inset shows AFM image of 3 cycle layer of poly-PATT.

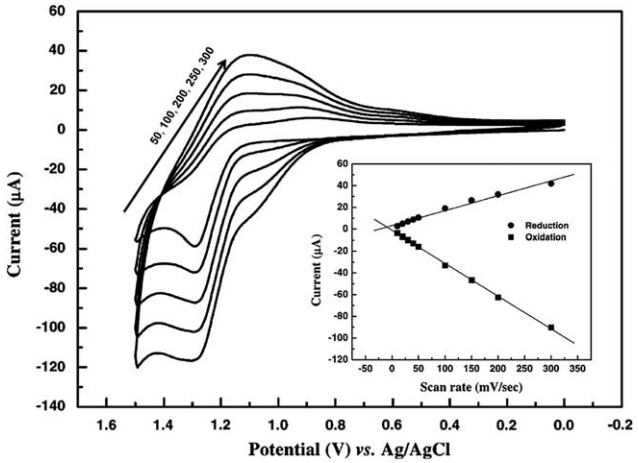

Fig. 3 CVs recorded for the poly-PATT film in a 0.1 M TBAP dichloromethane solution at various scan rates and scan rate increases from 50, 100, 200, 250, 300 mV s^{-1}.

with generation of quinine. The catalyzed reactions on the electrode surface are

$$\text{Catechol} + \text{tyrosinase}(O_2) \rightarrow o\text{-quinone} + H_2O \quad (1)$$

$$O\text{-quinone} + 2H^+ + 2e^- \rightarrow \text{catechol (at electrode)} \quad (2)$$

Here, there was no need to use any electron mediators.

The presence of AuNPs in the interior of the dendrimer was proved by CV in a 0.1 M H_2SO_4 solution. The potential was scanned between −0.2 and +1.4 V at a scan rate of 0.1 V s^{-1}. An Au reduction peak was observed at +0.85 V *versus* Ag/AgCl in the case of the Poly-PATT/Den (AuNPs) electrode, which was not observed in the case of Poly-PATT/Den, clearly indicating that AuNPs successfully formed in the interior of dendrimers because the surface terminal COOH groups in the dendrimer have a negative charge, which repels the negative charged AuNPs (data not shown). On the other hand, AuNPs can be formed inside the dendrimer, because the tertiary amine groups inside the dendrimer can interact strongly with AuNPs. The electrocatalytic behavior of Poly-PATT/Den (AuNPs)/tyrosinase was tested for the catechol detection and found to be successful. However, we did not perform the experiment to verify the various sets of modified electrodes because Rahman *et al.*[25] investigated this by using HRTEM techniques and suggested that for the AuNPs with and without a dendrimer template, the size of the AuNPs was much higher (15–20 nm) than those prepared with a dendrimer template. The diameter of the dendrimer was about 3.6 nm (third-generation PAMAM dendrimer), and from the HRTEM image, they confirmed that the particle size of the AuNPs prepared with a dendrimer template

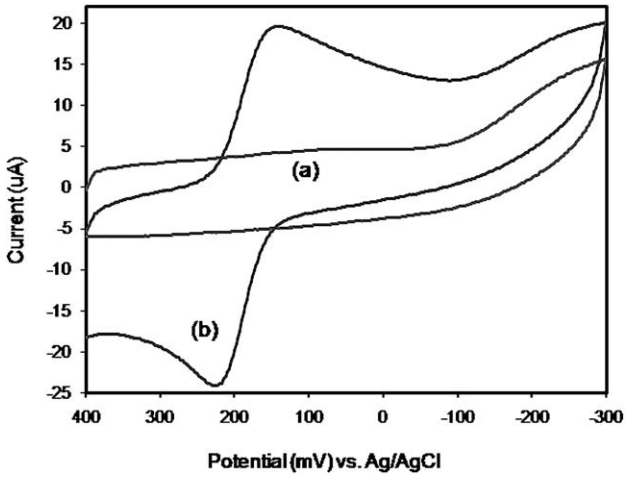

Fig. 4 CVs obtained at GCE modified nanobioelectrode (a) without catechol (b) with 0.1 mM catechol in a 0.1 M PBS (pH 7.0), scan rate: 50 mV s^{-1}.

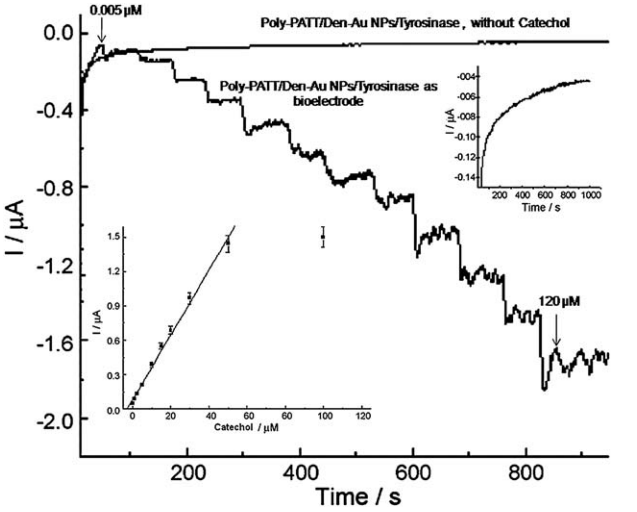

Fig. 5 Chronoamperometric measurements by successive addition of catechol solution into PBS at pH 7.0 solution containing catechol at applied potential +0.2 V *vs.* Ag/AgCl. Inset (bottom left) shows the calibration plot for catechol detection. Inset (top right) shows the blank noise level response.

was 1.7–2.0 nm, which clearly indicates that the AuNPs particle formed inside the dendrimer. They also confirmed the presence of AuNPs by electrochemical impedance spectroscopy (EIS), QCM, and XPS analysis. Fig. 4 shows the CV of the bioelectrode response in the presence and absence of catechol in PBS solution, at scan rate 50 mV s^{-1}. Fig. 4 shows CV recorded for a Poly-PATT/Den(AuNPs)/tyrosinase modified electrode in a PBS solution containing 0.1 mM catechol (b) and without catechol (a).

3.1 Analytical performance of bioelectrodes

The pH plays an important role in the sensing efficiency of the biosensor. The sensing efficiency increases with increasing pH value from 4.0 to 8.0 and then decreases with increasing pH value in a PBS buffer solution containing catechol. It can be seen that the current increased as the pH changed from 5.0 to 7.0, following by a large decrease in the pH range of 7.0–9.0. The maximum response was obtained at pH 7.0 and suggested that the immobilization procedure did not alter the inherent properties of tyrosinase. Thus, pH 7.0 PBS was used as the electrolyte in subsequent experiments. The response increases when the temperature increases from 10 to 30 °C, but no additional significant changes were observed between 30 and 50 °C. Thus, the optimal temperature for the detection of catechol was chosen as 30 °C. At high temperatures, the amperometric sensitivity of the biosensor for catechol is sharply decreased due to the partial denaturation of the immobilized tyrosinase. In addition, the effect of applied potential on the detection of catechol was examined in a chronoamperometric experiment. The current response increases when the applied potential varies from +0.4 to −0.3 V. The maximum response was observed at +0.2 V and the application of more negative potentials up to −0.3 V, which did not increase the current response. Thus, the bionanoelectrode was polarized at +0.2 V in subsequent amperometric experiments.

The amperometric response of the proposed biosensor was examined by successively increasing the concentration of catechol under the optimized conditions. The result is shown in inset of Fig. 5. A linear relationship between current and the concentration of catechol was observed with a correlation coefficient of 0.999 when the concentration of catechol is between 0.005 μM and 120 μM. The detection limit was around 0.002 μM, which was calculated from a signal-to-noise ratio of 3. The analytical performances of the proposed biosensor were compared with other tyrosinase biosensors reported in the literatures. The linear response range and the limit of detection of the biosensors are all described in Table 1 and suggest that the proposed nanobiosensor exhibited improved analytical performances in terms of linear range and limit of detection when compared with other reported biosensors. Thus, the developed electrochemical biosensor is an excellent candidate for the detection of catechol. To demonstrate the selectivity of our sensor, the interferences of typical organic common phenolic compounds, such as phenol, *m*-cresol, 3-aminophenol, 4-nitrophenol and 2,4-dinitrophenol, were also examined during the amperometric response for catechol. 20 μM catechol and 100 μM interference compounds were added into solution each time (data not shown). The influences of these existing species on the current responses of catechol were negligible or did not interfere in catechol detection. The potential of catechol on our electrode was lower, so some electroactive phenolic compounds did not significantly appear at this potential. The coexisting species had 5 times the concentration of catechol, but still no interference was observed in the determination of catechol. This indicates that our bioelectrode has high selectivity toward the detection of catechol at this low potential. The response time of the fabricated sensor to catechol was less than 7 s at a concentration of 20 μM.

Fig. 5 shows the current–time plot after the addition of catechol in a PBS solution. The successive additions of catechol at concentrations 0.005–120 μM were used and the oxidation current rose steeply to a stable value. The modified electrode achieved 95% of steady-state currents within 7 s. Fig. 5 inset shows the calibration plot for catechol detection. Under the

Table 1 Comparison with other catechol biosensors based on tyrosinase

Type of electrode	Linear range	Detection limit/nM	References
Colloidal gold nanoparticles/ graphite-Teflon/tyrosinase	0.010–8.0 M	20	27
Tyrosinase/3-mercaptopropionic acid-modified Au electrodes	0.2–100 M	88	19
MWNT-Nafion-Tyr nanobiocomposite film modified glassy carbon electrode	1–23 μM	130	26
Organoclay-enzyme film electrodes	0.2–15 M	—	28
Sol–gel immobilized tyrosinase electrode	1–60 M	200	29
Nafion/ZnO/tyrosinase films	0.01–0.4 mM	4000	30
Nano-HA/chitosam/tyrosinase modified Au electrode	10nM to 7 M	5	31
Poly3,4-ethylenedioxithiophene/ tyrosinase electrode	—	5	32
Poly-PATT/Den(AuNPs)/ tyrosinase	0.005–0.120 μM	2	Present work

optimized condition, the steady-state currents showed a linear relationship with the catechol concentration in the range of 0.005–120 μM. This linear dependency of the catechol concentration yields the regression with correlation coefficient of 0.997. The proposed nanobiosensor exhibits a good hydrodynamic range at applied potential +300 mV to −300 mV (data not shown). Further, experimental study shows that this nanobiosensor exhibits high stability, when it was stored in a desired buffer solution at refrigerated conditions for a period of 2 months, and the nanobiosensor retained more than 95% of its initial response to the catechol. The bioelectrode lost only 5% of the initial response in about five continuous measurements.

Table 2 Determination of catechol in real water samples ($n = 3$)

Real water souces	Catechol added/μM	Catechol found/μM	Recovery(%)
Milli Q water	10	9.4	94.0
	25	23.0	92.0
	50	47.5	95.0
Drinking water	10	9.1	91.0
	25	23.1	92.4
	50	47.3	94.6
Tap water	10	8.94	89.4
	25	23.5	94.0
	50	47.4	94.8

3.2 Determination of catechol in real water samples

To study the real possibility of the proposed biosensor we tested the proposed biosensor in the determination of catechol added to a matrix of real water samples. The water samples were obtained from the laboratory (South Korea). The results of analysis obtained for different types of water are presented in Table 2. Milli Q water samples indicated a relatively better quality of water source. This study was performed by adding known amounts of standard catechol solutions to the real water samples (10, 25 and 50 μM) followed by analysis using the proposed biosensor. Satisfactory values between 85 and 95% for catechol were obtained for the recovery, thus indicating that there is no significant interference from the various sample matrix in the proposed method and also the result of the catechol biosensor clearly indicates that the biosensor can be used in real wastewater samples for the detecting submicromolar concentration of catechol.

4. Conclusions

In the present work, an electrochemical nanobiosensor for the detection of catechol was constructed using the enzyme tyrosinase immobilized onto a GCE modified nano-composite matrix. The analytical characteristics of this nanobiosensor, including linear range, and lower detection limit have been described. The nanobiosensor exhibited good performance in terms of reusability, operational stability, fabrication simplicity, and shelf life. The proposed nanobiosensor was targeted for the detection of catechol in real water samples. The above observations showed that the developed nanobiosensor might be promising in the detection of catechol in real water samples at nanomolar concentration.

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