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Direct electrochemistry of alcohol oxidase using multiwalled carbon nanotube as electroactive matrix for biosensor application

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ABSTRACT

Rapid detection of alcohol is important in clinical diagnosis and fermentation industry. An octameric alcohol oxidase (AOx) (Mr 675 kDa) from *Pichia pastoris*, immobilized on multiwalled carbon nanotubes—Nafion® (MWCNT-Nf) matrix and encapsulated with polyethylenimine (PEI) on gold electrode (AuE), showed a redox peak at 0.21 V (vs. Ag/AgCl electrode at pH 7.5) for oxidation of alcohol. The electron transfer rate constant and surface coverage of the immobilized AOx were $1.69 \pm 0.15\ s^{-1}$ and 2.43×10^{-12} mol cm^{-2}, respectively. Studies on response and kinetics of Au-MWCNT-Nf-AOx-PEI bioelectrodes for alcohol showed a linear response in the range of 8 μM–42 μM, response time of 55 s for steady state current, and detection limit of 5 μM. The bioelectrode retains ~90% of the original response even after four weeks when stored in potassium phosphate buffer pH 7.5 at 4 °C. The fabricated bioelectrode was found to exclude interference caused by the common electroactive species such as ascorbic acid, uric acid, lactic acid, glucose and urea. The bioelectrode also showed reliable response characteristics in blood serum samples. The findings of the investigation have established the direct electrochemistry of the AOx protein and its potential biosensor application for quantitative detection of alcohol in blood serum.

1. Introduction

The detection and quantification of alcohols with high sensitivity, selectivity and accuracy are required in many different areas, such as, clinical and forensic analysis, food, beverage, and pulp industries [1]. Many analytical methods based on chemical, chromatographic, and spectroscopic principles have been developed for the determination of ethanol. Although, a few of these methods are reliable, they are complex, time consuming and require prior separation processes, expensive instrumentation and trained operators. Such disadvantages could be overcome by using biosensor devices incorporating the enzymes as bio-recognition elements [2]. Biosensors reported so far for quantitative detection of alcohol are yet to develop properly for commercial applications. Alcohol biosensors described in the literature are largely based on alcohol dehydrogenase (ADH) [3], which normally require the presence of co-factor nicotinamide adenine dinucleotide (NAD^+) [4–7]. The major constrain for developing ADH based alcohol biosensor is the need of supplementing the co-factor (NAD^+) to the reaction medium [8]. Although there is a continuous effort to regenerate the co-factor in the reaction system to sustain the catalytic activity of ADH, efficient cofactor regenerating system for biosensor application of ADH is yet to develop properly. Alcohol oxidase (AOx) (EC 1.1.3.13) as bio-recognition element for sensing alcohol has stimulated interest since the last decade [1]. Some potential advantages of AOx based bio-recognition system are identified among which the irreversible catalytic oxidation of alcohols and the avidly bound flavin-based co-factor to the redox center of the enzyme are pertinent to the biosensor applications. Moreover, the knowledge on molecular and functional characteristics of this unexplored group of redox enzymes is rapidly growing since recent past. AOx catalyzes the oxidation of alcohols using molecular oxygen as the electron acceptor and producing corresponding carbonyl compound and hydrogen peroxide in the reaction medium. The reaction may be followed by measuring O_2 or H_2O_2 concentration using optical or electrochemical detections [1,8–16]. The oxygen-based detection, however, has practical inconveniences and limitations such as, poor response, low accuracy and reproducibility, and high background signal that may raise the minimum detection level of the substrate alcohol [17]. Nevertheless, H_2O_2 based sensor has the advantages of the relative ease of manufacturing and the possibility of constructing them in small sizes, the high potential (~600 mV) necessary to oxidize H_2O_2 poses a problem of electrochemical interference

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due to the presence of reducing compounds (e.g. ascorbic acid, uric acid, bilirubin and acetaminophen), particularly in blood serum sample. Moreover, the response of the H_2O_2 based sensor is slow. The second generation AOx based amperometric biosensors using electron transport mediator that shuttles electrons between the redox center of the enzyme and the electrode are also not known so far since no suitable mediator has yet been found for AOx.

We report here an amperometric biosensor using direct electrochemistry of the large multimeric AOx from *Pichia pastoris* immobilized in a nano-composite matrix on the surface of gold (Au) electrode. Multiwalled carbon nanotubes (MWCNT), which have been used for fabricating bioelectrodes of many small enzymes [18,19] due to its good electrical conductivity, high mechanical strength, and antifouling property [20], were dispersed on the surface of the electrode using hydrophobic polymer chain of Nafion (Nf) [21]. Polyethylenimine (PEI), a polycationic polymer, was used to stabilize the AOx immobilized on the nano-matrix coated on an Au electrode [22]. The fabricated AOx bioelectrode has been characterized and detailed account on the response of the bioelectrode for the substrate alcohol is presented here.

2. Experimental

2.1. Chemicals and reagents

AOx from *P. pastoris* (21 U/mg protein), multiwalled carbon nanotube (MWCNT, size OD 10–15 nm, ID 2–6 nm, length 0.1–10 μm), Nafion117 (Nf) (5% w/v in isopropanol), polyethylenimine (PEI) (50% w/v in water solution), ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)], and horseradish peroxidase (HRP) were bought from Sigma-Aldrich (USA). Hydrogen peroxide (H_2O_2) 50% and Ethanol 99.9% was purchased from Merck. All other chemicals were of analytical grade and used as received without purification. AOx (1 mg ml^{-1}) was freshly prepared in potassium phosphate buffer solution (KPBS) (50 mM, pH 7.5).

2.2. Fabrication of the bioelectrode

A gold electrode (AuE) (diameter,1 mm) was cleaned first by polishing with alumina slurry, then washed ultrasonically with 70% ethanol and water separately for 5 min each and finally allowed to dry under clean air at room temperature. A total of 3 mg MWCNTs were dispersed in 300 μl of 5% Nf and sonicated for 20 min to obtain a stable homogeneous suspension. Five microliters of the MWCNT-Nf solution was dropped onto the AuE and then allowed to dry under clean air and room temperature. Once dried, 5 μl of AOx solution from the stock solution was dropped above the MWCNT-Nf layer, dried and covered with 5 μl of 10% (v/v) PEI solution. The fabricated bioelectrode was again dried under clean air at room temperature and stored in KPBS at 4 °C when not in use.

2.3. Apparatus and measurements

Cyclic voltammetry (CV) was performed with a potentiostat (Autolab PGSTAT 1212, Eco Chemie, Netherland). The measurement was done in a three-electrode system containing platinum rod as counter electrode, Ag/AgCl (saturated KCl) as reference electrode, and AuE or modified AuE as the working electrode. All potentials were measured and reported relative to the Ag/AgCl reference electrode. During the CV measurements, the electrolyte solution was constantly purged with high purity argon gas to maintain an anaerobic environment in the cell during the measurement process. All experiments were performed at room temperature. The images of the morphological characteristics on the bioelectrode were obtained on a field emission scanning electron microscope (FESEM) (Zeiss), with EHT 1.00 kV.

3. Results and discussion

3.1. Morphological characterization of the bioelectrode

The surface morphology of bare Au, Au-MWCNT-Nf, Au-MWCNT-Nf-AOx and Au-MWCNT-Nf-AOx-PEI bioelectrodes was investigated using FESEM. As shown in Fig. 1, AuE shows homogenous surface (Fig. 1A), and the Au-MWCNT-Nf electrode shows uniform distribution of MWCNTs on the electrode surface (Fig. 1B) with porous morphology clearly visible from the image taken at higher magnification (inset Fig. 1B). When AOx was added on this MWCNT-Nf film, the thread like structure of MWCNT and porosity of the film disappeared (Fig. 1C), indicating the immobilization of AOx molecules on the MWCNT-Nf film. Subsequently, when a layer of PEI was applied on the film to fabricate Au-MWCNT-Nf-AOX-PEI bioelectrode, the surface morphology changed to even form with an array of black dots in white patches distributed throughout the surface (Fig. 1D). The immobilization of enzymes on the electrode surface occurred under the influence of electrostatic interaction between the negatively charged underneath MWCNT-Nf layer with adsorbed AOx and positively charged overlaying PEI layer. The negative charges on MWCNT and the sulphonated polymer, Nf are well described. The AOx (p*I* 4.3) bears negative charge under the working buffer pH value of 7.5 used in this investigation. The formation of the nearly uniform array of black dots upon encapsulation of the AOx with PEI is interesting. The probable reason being attributed to the reorientation of the negatively charged MWCNTs in the composite matrix to the arrays upon its interaction with the positively charged PEI. However, the role of AOx in the reorientation of the MWCNTs is not clearly known.

3.2. Electrochemical characterization of the bioelectrodes

CV was carried out to identify the redox potentials of the fabricated working bioelectrodes (Fig. 2) in a solution of argon-saturated KPBS (pH 7.5) at a scan rate of 50 mV s^{-1}. No clear redox peaks were observed for the bare AuE-Nf and AuE-MWCNT-Nf suggesting that both the MWCNT and Nf are electro-inactive in this potential window. Following the immobilization of AOx in the MWCNT-Nf film the generated bioelectrode AuE-MWCNT-Nf-AOx displayed a pair of clear redox peaks. However, the bioelectrode AuE-Nf-AOx-PEI (Supplementary Fig. 1) showed no clear redox peak and the CV response of the MWCNT void bioelectrode was almost similar with Au-Nf, which infers that MWCNTs play an important role in facilitating the electron exchange between the redox center of AOx and AuE. When PEI was layered on the AuE-MWCNT-Nf-AOx electrode the redox peak current of the generated bioelectrode AuE-MWCNT-Nf-AOx-PEI was increased further. The formal potential of the redox couple at the bioelectrode AuE-MWCNT-Nf-AOx-PEI calculated from the average value of anodic and cathodic peak potentials $[(E_{pa}+E_{pc})/2]$ was 0.21 V. The formal potential confirmed from the triplicate studies was 0.21 ± 0.003. The separation of the peak potentials (ΔEp) was 60 mV. The anodic peak current is nearly equal to the cathodic peak current. Therefore, a direct electrochemical reaction of quasi-reversible is ascribed to AOx immobilized on MWCNT-Nf-AOx-PEI. The potential of 0.21 V is attributed to the redox potential of $FAD/FADH_2$ system. Protein-bound flavins exhibit wide range of redox potentials since the redox potential of flavo-oxidases is strongly modulated by the cofactor environment [23,24]. The redox potential of AOx was further confirmed by the increased current response at the potential with the addition of substrate alcohol. From the above findings it may be suggested that the nanometric MWCNT may be easily accessed to the multimeric AOx protein molecules, as a result of which the distance between the electrode and the redox center of AOx was decreased and promoted the electron exchange between them similar to other enzyme nanomaterial integrated system [25]. The increase in redox current at 0.21 V of the PEI modified bioelectrode AuE-MWCNT-Nf-AOx-PEI is attributed to the electrostatic effect of PEI on stabilizing the AOx in the electrode, which

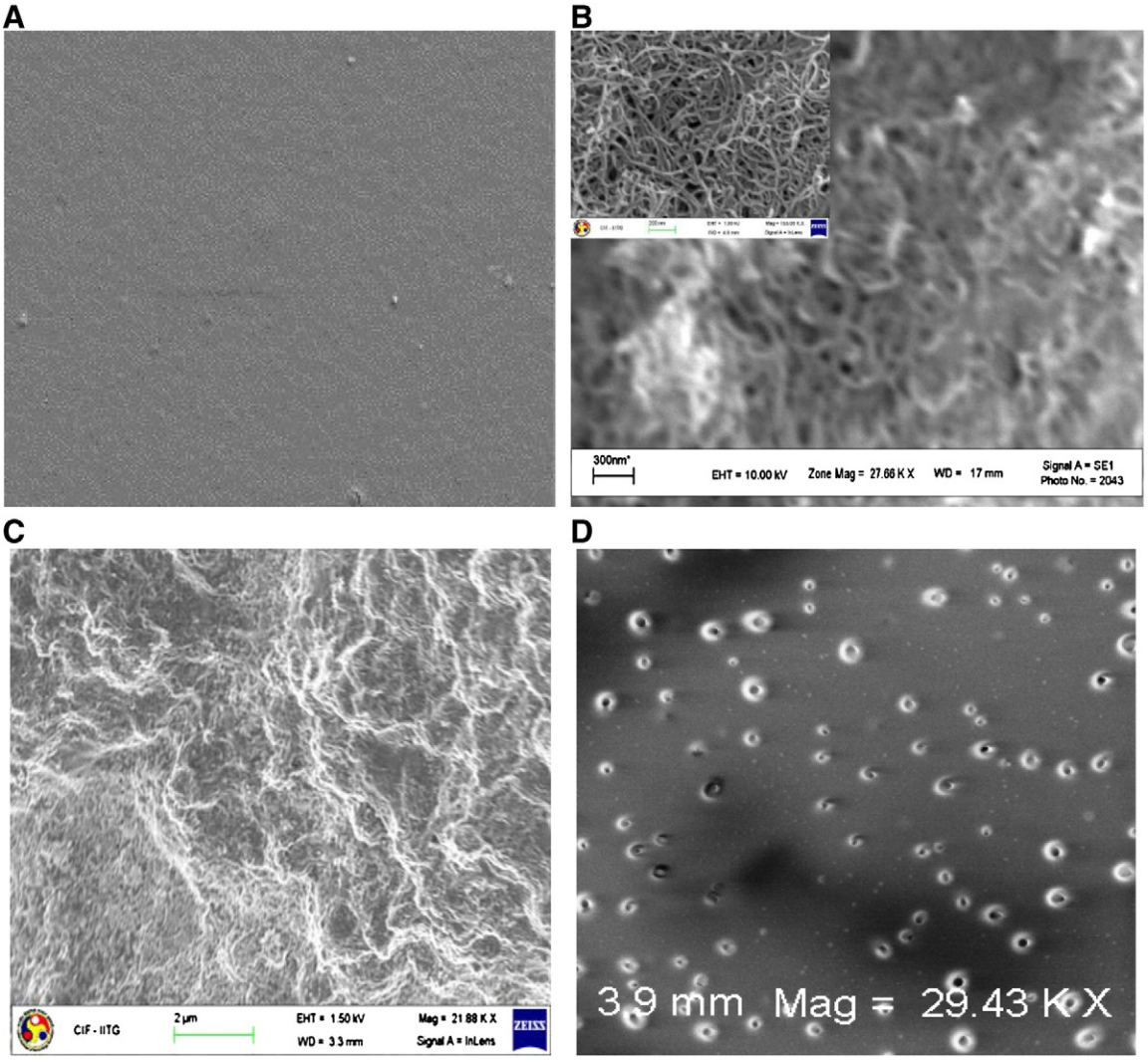

Fig. 1. FESEM images of (A) AuE (30 KX), (B) Au-MWCNT-Nf (27.66 KX) with inset at 153 KX, (C) Au-MWCNT-Nf-AOx (21.88 KX) and (D) AuMWCNT-NF-AOx-PEI (29.43KX) with EHT 1.00 kV.

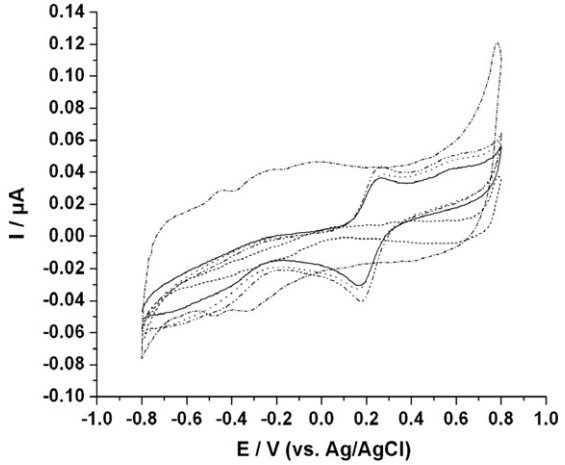

Fig. 2. CV of AuE-Nf (-----), AuE-MWCNT-Nf (_ _), AuE-MWCNT-Nf-AOx (___), AuE-MWCNT-Nf-AOx-PEI (.....) and AuE-MWCNT-Nf-AOx-PEI (_ . _ . .) with 33 µM ethanol in an argon gas purged solution of 0.1 M KPBS (pH 7.5) at a scan rate of 50 mV s^{-1}.

enhanced the interaction between the electroactive center of the AOx proteins with the MWCNTs. The interaction further facilitated the electron transfer from the redox center of the enzyme to the electrode surface.

The electrochemical behavior of the constructed bioelectrode was characterized by CV at different scan rates (ν) (Fig. 3A). The anodic (I_{pa}) and cathodic (I_{pc}) peak currents versus scan rate plots, shown in Fig. 3B, exhibit a linear relationship with a correlation coefficient of 0.9734 and 0.9877, respectively which infers that the redox reaction of AOx is a surface-controlled process, not a diffusion controlled process. The values of anodic and cathodic peak potentials were plotted against the logarithm of the scan rates as shown in Fig. 3(C). The irreversible electrochemistry (i.e., peak separation $>200/n$ mV taking $n=1$) occurred when the scan rate exceeded 0.7 V s^{-1}. The electron transfer coefficient (α) and heterogeneous electron transfer rate constant (k_s) for diffusionless thin-layer voltammetry were determined from empirically fitted Laviron Eq. [26]. The anodic (E_{pa}) and cathodic (E_{pc}) peak potential at various scan rates are expressed as Eqs. (1) and (2):

$$E_{pa} = E^{o'} + 2.3RT/(1-\alpha)nF \log \nu \quad (1)$$

$$E_{pc} = E^{o'} - 2.3RT/\alpha nF \log \nu \quad (2)$$

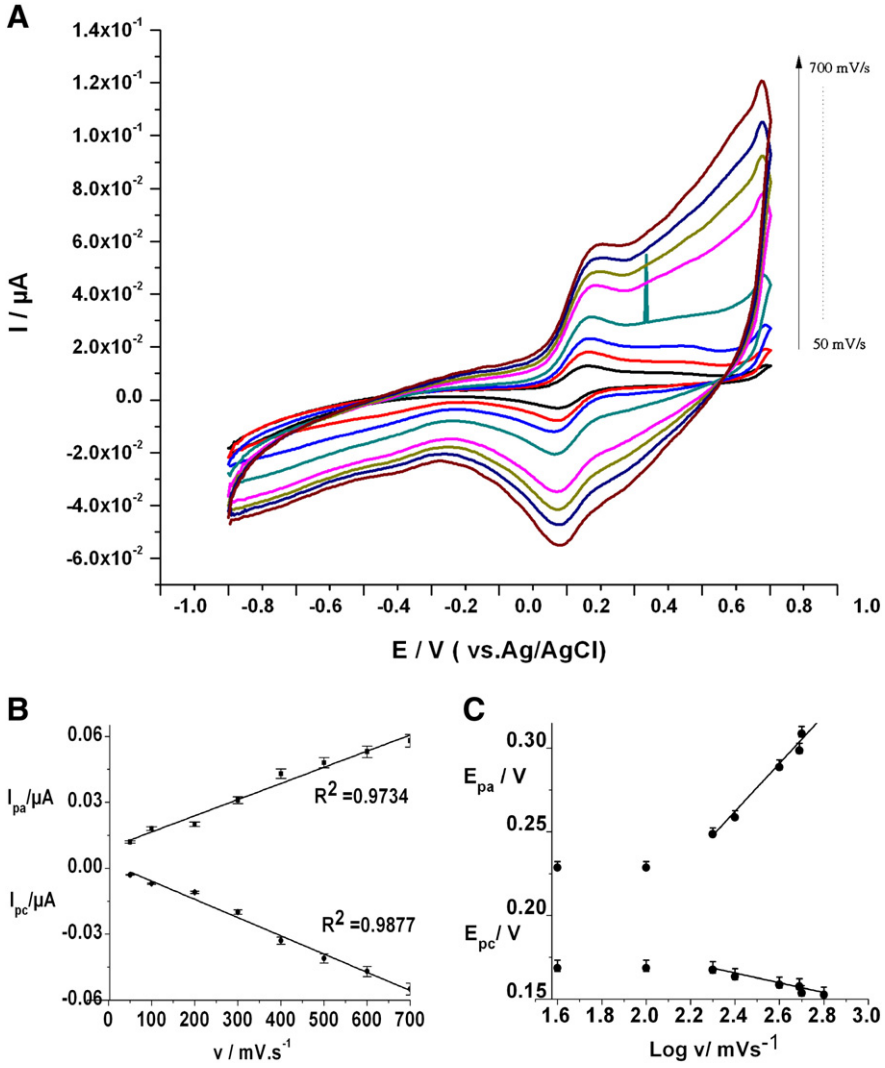

Fig. 3. (A) CV of bioelectrode AuE-MWCNT-Nf-AOx-PEI with increasing scan rate (mV s^{-1}) (50, 100, 200, 300, 400, 500, 600, 700); (B) anodic (I_{pa}/µA) and cathodic peak current (I_{pc}/µA) versus scan rate (mV s^{-1}). I_{pa}/µA = $(7.41\times10^{-5}\pm1.7\times10^{-6})$ (v)/(µA.mV.s^{-1}) + $(8.09\times10^{-4}\pm1.89\times10^{-4})$/(µA). ($R^2$ = 0.9734) and I_{pc}/µA = $(-7.43\times10^{-5}\pm2.6\times10^{-6})$ (v)/(µA.mV.s^{-1}) + $(0.008\pm5.7\times10^{-4})$/µA. ($R^2$ = 0.9877) (C) peak potential versus log (scan rate, mV s^{-1}) in a solution of 0.1 M KPBS, pH 7.5.

where $E^{o'}$ is the formal potential, and R, T, F, and n have their usual significance. According to Eqs. (1) and (2), the value of α was determined from the relationship of ΔE_P with log v and found to be 0.52 (taking $n = 1$). The value of k_s was calculated using Eq. (3) and found to be 1.69 ± 0.15 s^{-1}

$$\log(k_s) = \alpha\log(1-\alpha) + (1-\alpha)\log\alpha - \log RT/nFv - \alpha(1-\alpha)nF\Delta E_P/2.3RT. \quad (3)$$

The result suggests significant electron transfer between the AOx and the electrode. There is however, no previous report on these kinetic constants from AOx based bioelectrode for comparison in quantitative scale. The surface coverage (Γ) of AOx on the electrode surface at a scan rate of 50 mV s^{-1} estimated using the equation, $\Gamma = Q/nFA$ [27], where Q is the charge obtained by integrating the peak current area, n is the number of electrons involved, F is the Faraday's constant and A is the electrode area, was found to be 2.43×10^{-12} mol cm^{-2}. The amount of this electroactive protein is nearly 80% of the total amount of AOx deposited on the electrode surface. This may be suggested that only those proteins with a suitable orientation and close contact with the MWCNTs in the inner layers of the film can exchange electrons with the gold electrode. Theoretically, the maximum possible electrode surface coverage with a monolayer of the AOx protein calculated from the molecular dimension of AOx [28] was 1.25×10^{-4} mol cm^{-2}. Comparing this theoretical value with the one reported by us it may be inferred that there is scope for optimizing the concentration of electroactive AOx molecules on the electrode surface by increasing the enzyme loading on the nanomatrix.

3.3. Response characteristics of the bioelectrode

The response of the bioelectrode was studied at various pH (6–8) values of the KPBS at equal concentration of alcohol (8 µM) and identified pH 7.5 as optimum for the response. It is known that the stability of AOx decreases when the pH is deviated from the physiological pH [29]. The optimum pH value for the biocatalytic reaction of the AOx was also 7.5 which conformed to other reports [30]. The pH effect on the alcohol biosensor observed here also conformed to other findings [31].

Initially we investigated the cyclic voltammetric response of AOx on the AuE-MWCNT-Nf-AOx-PEI in air saturated condition containing alcohol with increasing concentrations at a scan rate of 50 mV s^{-1} (Fig. 4). Similar pair of redox peaks as observed in the presence of argon purged solution was observed. However, as compared to the anodic peak current an increase in cathodic peak current at 0.15 V was observed in the air saturated solution. This is because the electrochemically formed

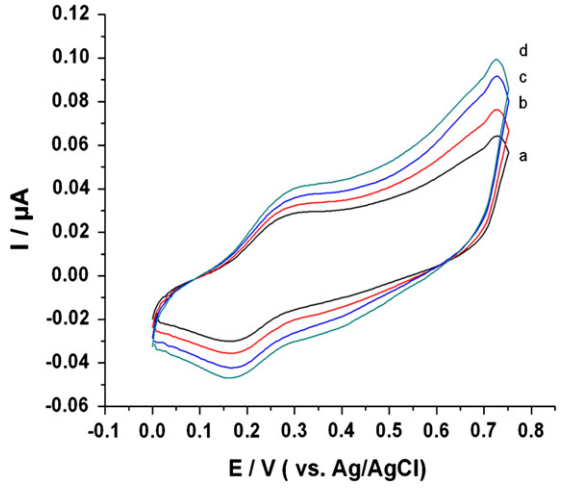

Fig. 4. CV of bioelectrode Au-MWCNT-Nf-AOx-PEI in the presence of oxygen saturated buffer (0.1 M KPBS pH 7.5) with increasing ethanol concentration (μM). (a) 0, (b) 8, (c) 33, and (d) 42 at a scan rate of 50 mV s^{-1}.

AOx ($FADH_2$) catalyzed the reduction of dissolved oxygen in the solution. A parallel increase in broad oxidation peak between +0.65 V and +0.7 V occurred due to the electrocatalytic oxidation of H_2O_2 produced by the biocatalytic oxidation of alcohol in oxygen containing electrolyte solution. These results infer that the AOx layered on the electrode film retains its bioactivity.

The amperometric response of Au-MWCNT-Nf-AOx-PEI bioelectrode for successive step changes of alcohol concentration in KPBS at the anodic potential of 0.27 V was measured (Fig. 5A). The magnitude of the anodic peak current was increased linearly with increasing ethanol concentration with a correlation coefficient of 0.9909 and reached saturation at 42 μM of ethanol (Fig. 5B). The response characteristics of the bioelectrode are summarized in Table 1.

The detection limit (DL) for the constructed biosensor was determined from the expression DL = 3 × SD / sensitivity (where SD is the estimated standard deviation for the points used to construct the calibration curve and sensitivity its slope). The sensitivity of our constructed bioelectrode with the direct electrochemistry compares favorably with AOx biosensor for hydrogen peroxide-based detection of total content of volatile alcohols reported recently [32].

3.4. Reproducibility, long-term stability and interference study of the bioelectrode

The operational stability of the fabricated bioelectrodes was investigated from sixteen successive measurements with 42 μM alcohol (which was the highest response in the linear range) during a period of 4 h. It was observed that the current response was steady till the last measurement following which the response declined. The results indicate that the activity of the constructed bioelectrode was quite stable during the period. The fabrication reproducibility of the bioelectrode was estimated from the response to various alcohol concentrations at three bioelectrodes prepared by similar procedure. The results showed acceptable reproducibility with a relative SD of ~2% for the bioelectrode. The storage stability of the bioelectrode was checked by carrying out response measurements at a regular interval of three days. It was found that the bioelectrode retains about 90% of the original response even after four weeks when stored in KPBS (pH 7.5) at 4 °C.

To study the selectivity of the alcohol biosensor to alcohol, the effect of some potential interfering agents present in real samples namely, ascorbic acid, lactic acid, glucose, urea and uric acid, on biosensor response was studied. Biosensor response was examined by exposing it separately to these interferents, each present with 8 μM alcohol solutions in 1:1 ratio. The selectivity coefficient (SC) of the biosensor for

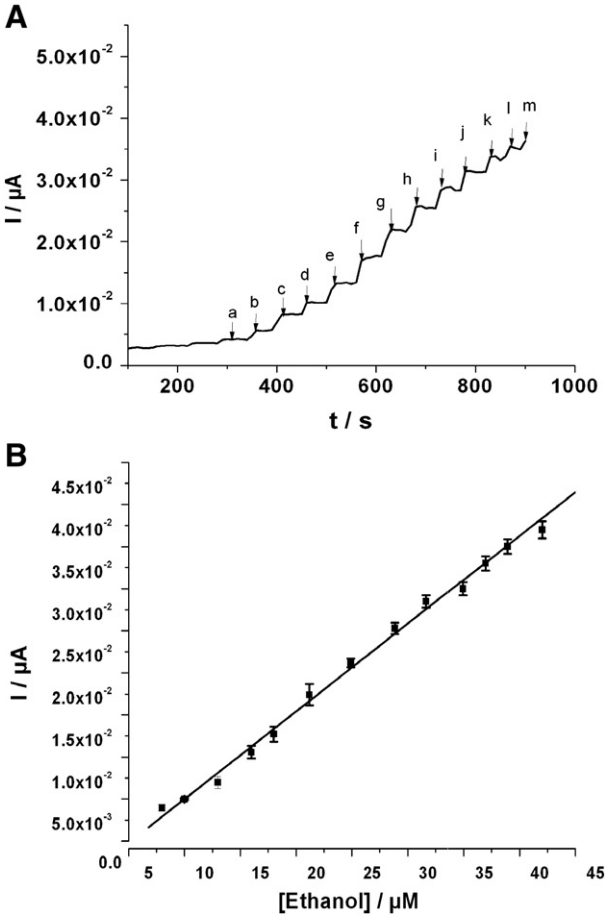

Fig. 5. (A) Chronoamperometric current responses of the AuE-MWCNT-Nf-AOx-PEI bioelectrode for successive addition of alcohol (μM). (a) 8, (b) 10, (c) 13, (d) 16, (e) 18, (f) 21, (g) 25, (h) 28, (i) 31, (j) 35, (k) 37, (l) 39, and (m) 42. (B) The response curve of the bioelectrode with increasing ethanol concentration in argon purged solution of 0.1 M KPBS, pH 7.5. I/μA = $(0.001 \pm 2.8 \times 10^{-5})$ [Ethanol]/μA $\mu M^{-1} + (0.0052 \pm 7.7 \times 10^{-4})$/μA ($R^2 = 0.9909$). The operating potential was 0.27 V (vs. Ag/AgCl electrode).

each interferent was estimated with respect to the response of the biosensor obtained when it is subjected to only alcohol using the formula, $SC = I_{c+1}/I_c$, where I_{c+1} and I_c [33] are biosensor responses for alcohol (8 μM) in the presence and absence of each interferent, respectively. It was found that the contribution of these compounds to the biosensor response is <5% implying no significant interference. The actual level of these interferents in blood serum is usually known to be very low as compared to the concentration used in this study. The optimum selectivity of the bioelectrode towards alcohol may be attributed to two factors. Firstly, the anodic potential chosen is 0.27 V. At such a low potential, the contribution of many interferents towards current response may get substantially minimized. Nafion polymer acts as a selective barrier for the restricted access of many interferents to the electrode surface by electrostatic repulsion [21]. This study implies that the biosensor could be used in the physiological conditions without any significant interference from the substances present in the body fluids. The practical usability of the fabricated bioelectrode was evaluated by

Table 1
Response characteristics of the bioelectrode.

Linear range	8 μM–42 μM
DL	5 μM
Sensitivity	3 ± 0.08 μA mM^{-1}
SD limit	$4.94 \times 10^{-3} \pm 0.08 \times 10^{-2}$ μA
Response time	55 s

Table 2
Comparison on performances of various AOx-based biosensors.

Biosensor configuration	Detection approach	Operational stability	Linearity (μM)	Reference
Carbon paste matrix/AO_x-HRP-osmium (FIA)	Hydrogen peroxide	10% of sensitivity lost after 270 injections carried out in 9 h	250–2000	Vijayakumar et al. [8]
Pt/AO_x-poly(carbamyl) sulfonate hydrogel (Batch)	Hydrogen peroxide and oxygen	32% of sensitivity lost after 12 measurements carried out in 12 h, and 40% of sensitivity lost after 18 days	10–3000	Patel et al. [9]
Pt/polyvinylferrocenium matrix-AO_x (Batch)	Hydrogen peroxide	98% of sensitivity lost after 36 days (132 measurements)	Up to 3000	Gulce et al. [10]
Carbon paste matrix-(graphite-Teflon)/AO_x-HRP-ferrocene (FIA)	Hydrogen peroxide	No loss after 15 days (3 measurements/day)	10–750	De Prada et al. [14]
Au/PPY_{ox}/AO_x-glutaraldehyde-BSA (FIA)	Hydrogen peroxide	No loss after 90 injections carried out in 3 h, 5% of sensitivity lost after 6 h online cont. monitoring	Not reported	Carelli et al. [12]
Chitosan/AO_x-eggshell membrane (Batch)	Oxygen	No loss after 20 measurements carried out in 8 h, 13.4% of sensitivity lost after 3 months	60–800	Wen et al. [31]
Carbon film electrode MWCNT/AOx	Hydrogen peroxide	A decrease of 70% of the initial sensitivity value after 3 weeks	1400	Gouveia-Caridade et al. [35]
Au-MWCNT-Nf-AOx-PEI	Direct electrochemistry	No loss after 16 measurements carried out in 4 h, it retains about 90% of the original response after 4 weeks when stored in KPBS	8–42	Present work

determining the alcohol content in human serum samples. The alcohol content in the four serum samples supplemented with alcohol was first estimated by measuring the production of H_2O_2 using a coupled AOx-peroxidase reaction with ABTS ($\varepsilon_{405} = 36.8\ mM^{-1}\ cm^{-1}$) in KPBS buffer, pH 7.5 [34]. Then, the same samples were analyzed electrochemically with the bioelectrode at 0.27 V after diluting the samples suitably with KPBS buffer to make the concentrations fall in the linear range of the bioelectrode. The data were compared by using paired t-test (Sigma 12). These results showed a reasonable agreement ($P = 0.045$) between both methods of analysis.

One of the major focuses of the AOx based biosensor is to prolong the functional activity of this complex multimeric enzyme on the electrode surface. Many AOx based first generation biosensors using hydrogen peroxide or oxygen based detection showed varying operational stability [8–16]. While the requirement of high over potential for the operation of first generation biosensors stands a stumbling block for developing interference free detection system, Gouveia-Caridade et al. [35] developed a H_2O_2 detection-based alcohol biosensor in which AOx and BSA were cross linked with glutaraldehyde and attached to the solid MWCNT on the carbon film electrode. The authors have reported significant increase in the sensitivity of the biosensor, while they reported its limitations on the reproducibility and poor operational stability. A comparative analysis, as shown in Table 2, infers that the method of fabrication of the bioelectrode is critical and linked to the stability and response mechanism of the biosensors. The electrostatic interaction principle exploited by us for the adsorption-based physical immobilization of the enzyme on the electrode surface provides the significant operational stability and the reproducibility on the response of the biosensors. Further, the interaction facilitates the electrical communication between the AOx with the electrode as evident from the clear CV response peak at comparatively low over potential of 0.21 V. Although the linear response range obtained by us is comparatively narrow the minimum detection limit in the linear range was superior to other reports mentioned above.

4. Conclusion

The direct electrochemistry of a multimeric alcohol oxidase (AOx) physically immobilized by encapsulating with PEI in a MWCNT-Nf matrix on the gold electrode surface was established for the first time. Direct electron transfer facilitated by the MWCNT between the AOx protein and the electrode was confirmed. The entrapped AOx possesses good bioactivity and electrocatalytic activity at room temperature and physiological pH. The constructed bioelectrode showed linear response at the oxidation potential of 0.27 V for alcohol. The values on operational and storage stability and detection limit in the linear response range obtained by using the bioelectrode towards the substrate alcohol were comparable to or even improved than many reports on alcohol biosensors. The low over potential of the constructed biosensor practically nullifies the interference caused by many common interfering compounds present in the samples. The findings confirmed the potential applications of the constructed bioelectrode as third generation biosensor for detecting alcohol in real samples.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bioelechem.2012.08.007.

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