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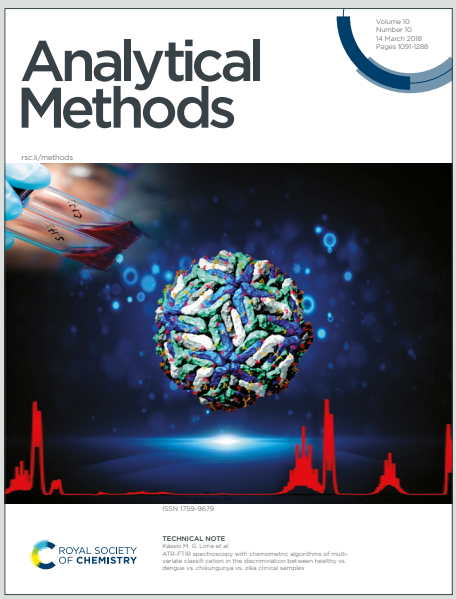

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ARTICLE

Disposable biogenic amine biosensors for histamine determination in fish

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This study presents the development of disposable biosensors employed in the determination of histamine in fish sample. Screen printed carbon electrodes (SPCE) were first modified with a mixture of titanium dioxide nanoparticles (TiO_2), carboxylated multiwalled carbon nanotubes (c-MWCNT), hexaammineruthenium (III) chloride (RU) and chitosan (CS). Diamine oxidase (DAO) or monoamine oxidase (MAO) enzymes were further immobilized onto TiO_2–c-MWCNT–RU–CS/SPCE via 1-ethyl-3-(dimethylaminopropyl) carbodiimidehydrochloride (EDC) and hydroxysuccinimide (NHS) chemistry for the fabrication of the biosensors. The morphological and electrochemical properties of the proposed biosensors were studied using scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), cyclic voltammetry (CV), chronoamperometry and electrochemical impedance spectroscopy (EIS). A performance comparison of two biosensors indicated that the one based on DAO had a linear concentration range from 9.9×10^{-6} – 1.1×10^{-3} M and the other based on MAO from 5.6×10^{-5}–1.1×10^{-3} M for histamine. The sensitivity of the DAO based biosensor was almost 1.5 times higher than that of the MAO based biosensor. The proposed biosensors were successfully employed to determine histamine in fish sample and the recoveries were between 100.0% and 104.6%.

Introduction

Biogenic amines (BAs) are low molecular weight aliphatic, alicyclic or heterocyclic organic bases[1]. Biogenic amine formation can occur during food processing and storage as a result of bacterial activities and they can be used as chemical indicators of food quality[2]. Among various BAs, histamine, putrescine, cadaverine and tyramine are found to be the most important BAs related with food spoilage[3]. However, histamine is one of the most biochemically active compounds from these BAs and it causes histamine fish poisoning without changing the normal appearance and odor of the fish[4]. Histamine (or scombroid) fish poisoning is a food borne intoxication which can cause negative health effects such as headaches, skin rash, diarrhea, vomiting, hypotension and digestive problems[5,6]. Therefore, the level of histamine in food needs to be strictly controlled due to its toxicological risk and importance in food quality. The statutory limit of histamine in fresh fish samples indicated by the Commission Regulation (EC) 2073/2005 is 200 mg kg^{-1}, and similar limits are adopted by numerous countries[7,8]. For these reasons, development of rapid, economical, accurate and practical methods for histamine determination is becoming increasingly demanded. Among the several techniques proposed for determination of histamine such as gas chromatography[9,10], capillary zone electrophoresis[5], thin layer chromatography[11] and HPLC[12], electrochemical biosensors are cost effective, simple, highly sensitive, rapid and specific[4,13-16].

Carbon nanotubes (CNTs), high aspect ratio allotropes of carbon, have attracted considerable interest in biosensor research since their discovery in 1991[17] because of their unique electrochemical properties including large surface area, high chemical stability and good mechanical strength[18]. These outstanding properties make carbon nanotube based biosensors good alternatives for ultra-sensitive biosensing systems. Comparing with the conventional solid electrode based biosensors, the CNTs based biosensors show great advantages such as higher sensitivity and stability, short response time, lower detection limit and longer life time[19,20]. Such improved performance properties have provoked the research interest in the applications of CNTs as components for biosensors. Besides CNTs, TiO_2 nanoparticles, among other metal oxide nanoparticles are also being extensively used in biosensor fabrication due to their porous structure, high specific surface area, biocompatibility, chemical stability and photosensitivity[21,22]. Nowadays, the integration of CNTs and TiO_2 nanoparticles as modification matrix for sensor and biosensor fabrication have gained growing interest since these materials possess characteristics of the individual components with a synergistic effect[23-25].

The biosensors developed for the determination of BAs are generally based on the use of amine oxidase enzymes. These

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enzymes catalyze the conversion of the biogenic amine to corresponding aldehyde, NH_3 and H_2O_2 according to Equation 1[4].

$$RCH_2NH_2 + H_2O + O_2 \rightarrow RCHO + H_2O_2 + NH_3 \quad (1)$$

In these biosensors the consumption of O_2[26] or the generation of H_2O_2[15] can be monitored to quantify BAs. The determination of H_2O_2 requires the application of high potential where common electroactive species can act as interferences[27]. Thus, the determination of H_2O_2 at lower operating potentials has been intensively investigated[28,20]. The combination of amine oxidases with peroxidase enzymes[29] or the use of redox mediators[30] are the common strategies for the determination of H_2O_2 at lower potentials. Hexaammineruthenium chloride (RU) has been used as a mediator in the fabrication of biosensors due to its many advantages such as easy availability and stability in both oxidized and reduced forms. It also has a low redox potential which is important to eliminate the influence of easily oxidizable species[31,32].

Herein, we present two novel amperometric biosensors for histamine determination in fish samples. In order to investigate the effect of DAO and MAO enzymes on the performance of histamine biosensors, these enzymes were immobilized onto TiO_2–c-MWCNT–RU–CS modified SPCEs for the fabrication of the DAO/TiO_2–c-MWCNT–RU–CS/SPCE and MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensors. The proposed biosensors can combine the advantages of TiO_2 nanoparticles, c-MWCNTs and redox mediator RU and are expected to exhibit high analytical performance. The experimental parameters that affect the biosensor performance were optimized and performance characteristics of the biosensors were compared. The practical application of the biosensors to histamine determination in real sample was also addressed.

Experimental

Reagents and Equipments

Human recombinant MAO A (≥10 unit/mg protein, ≥3 mg protein/mL, EC 1.4.3.4.), DAO from porcine kidney (≥0.05 unit/mg solid, EC 1.4.3.22), histamine dihydrochloride, potassium chloride, cadaverine dihydrochloride, 2-phenylethylamine hydrochloride, spermine, spermidine, tryptamine, putrescine dihydrochloride, tyramine, boric acid, acetic acid, phosphoric acid, potassium hexacyanoferrate(III), potassium hexacyanoferrate(II) trihydrate, Nafion® perfluorinated resin solution, L-histidine, L-tyrosine, L-tryptophan, L-arginine, L-lysine, TiO_2 nanoparticles (< 100 nm particle size), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, N-hydroxysuccinimide, hexaammineruthenium (III) chloride and chitosan were supplied from Sigma–Aldrich. c-MWCNTs (outer diameter < 8 nm, length 10–30 μm) was from Cheap Tubes Inc. (Brattleboro, USA) and L-ornithine hydrochloride was from Acros Organics. Deionised water obtained from ELGA Purelab Option-S System was used throughout the experiments. SPCEs with working electrodes of 4 mm in diameter (model C110) were bought from Dropsens (Llanera, Spain).

Electrochemical measurements were performed with a Multi μStat 8000 Potentiostat/Galvanostat from Metrohm Dropsens (Llanera, Spain). The chronoamperometric measurements were recorded at −0.30 V with successive injections of standard biogenic amine solutions into a stirred solution of 0.05 M Britton-Robinson (BR) buffer. Optimum pH of each amine was used for the chronoamperometric measurements. Cyclic voltammograms were obtained in 0.10 M KCl solution containing 5.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) with a potential window from − 0.40 to + 0.80 V at 50 mV s^{-1} scan rate. SEM images were taken with a FEI, Quanta 450 FEG model scanning electron microscope. The EDX spectra was recorded using a Bruker detector.

Real samples preparation

Fish was obtained from a local market in the vicinity of the Faculty of Science campus of Ankara University and stored at +4°C in refrigerator. 2 g of previously homogenized fish sample and 5 mL of BR buffer solution (pH 7.5) were blended for 5 min. The blend was sonicated for 20 min, and then it was centrifuged for 10 min at 9000 rpm. The supernatant was diluted to 1:10 with BR buffer solution, being fish sample then suitable for analyses.

Biosensor preparation

CS solution (0.1 g/mL) was prepared according to the literature[33]. 19.5 mg of TiO_2, 23.6 mg of c-MWCNT and 20.0 mg of RU were dispersed in 1 mL of CS solution for 3 h under ultrasonic agitation. The histamine biosensors were fabricated as follows: 5 μL of TiO_2–c-MWCNT–RU–CS dispersion was dropped onto SPCE surface and allowed to dry at room temperature. EDC-NHS based amine coupling chemistry is a well-known method for the covalent bonding of enzymes onto carboxyl containing substrates. EDC-NHS activates the carboxyl groups to form N-hydroxysucciniimide esters. The latter promote the formation of amide bonds between the carboxyl groups on the substrate and the primary amine of the enzyme to be immobilized[34]. In our study, the modified electrode surface was activated by EDC-NHS to convert the carboxyl groups of the c-MWNTs into active carbodiimide esters. For this purpose, 3 μL of the mixture containing 25 mM EDC and 100 mM NHS was casted on the TiO_2–c-MWCNT–RU–CS/SPCE and left for approximately 20 min at room temperature to dry. Just before dryness was reached, 5 μL of DAO solution (1.0 U mL^{-1}) was pipetted onto the modified electrode and dried at + 4 °C for approximately 1 h to fabricate the DAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensor. In case of MAO, 5 μL of the enzyme (30 U mL^{-1}) was immobilized onto TiO_2–c-MWCNT–RU/SPCE according to the procedure described for DAO and the resulting electrode was denoted as MAO/TiO_2–c-MWCNT–RU/SPCE. The

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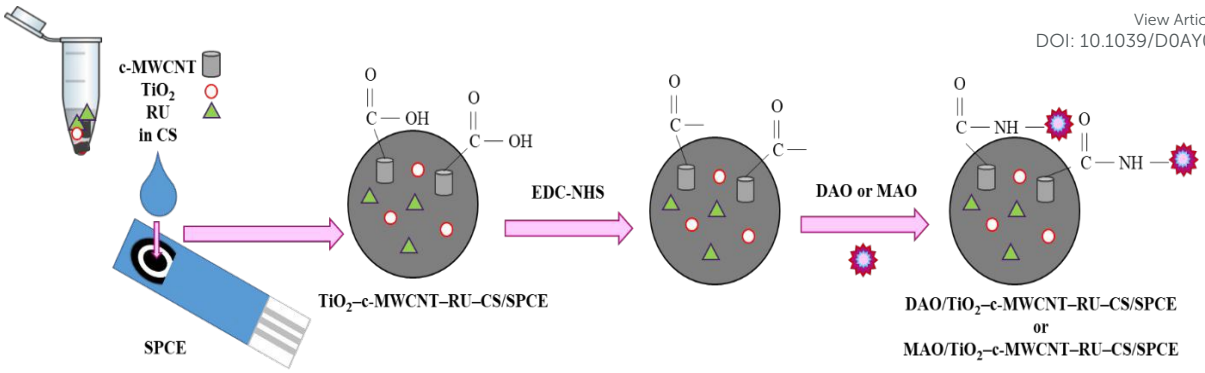

Scheme 1 The construction steps of the biosensors

enzyme immobilized electrodes were rinsed with 0.05 M BR buffer solution (pH 7.0) to remove the unbound enzyme. Finally, both electrode surfaces were further modified with 5 µL of 0.25% Nafion and the resulting biosensors were stored at + 4 °C when not in use. The procedure for the construction of the histamine biosensors is illustrated in Scheme 1.

Results and Discussion

Optimization of electrode modification

Optimization of electrode composition is an important point in the construction of modified electrodes. This can be achieved by the traditional one factor-at-a-time method which consists of changing the amount of one component at one time and keeping the amounts of other components constant. However, this approach is laborious and time-consuming. Central composite design (CCD) is another approach which can be used for the optimization of electrode modification. CCD is a combination of mathematical and statistical method and can be used for the rapid collecting of experimental data with minimum number of experiments. In our previous work, we have reported that the glucose biosensor fabricated using the optimum amounts of TiO_2 and c-MWCNT obtained from CCD showed higher sensitivity than the biosensor fabricated using the optimum amounts obtained from one factor-at-a-time method[25]. In this study, same TiO_2 and c-MWCNT amounts optimized before by 2^2 factorial CCD for the fabrication of a glucose biosensor were also used in the fabrication of the histamine biosensors. The response surface plots of the amperometric currents as function of c-MWCNT - TiO_2 is presented in ESI, Fig.S1. On the other hand, the amount of RU on the electrode surface can also affect the response of the biosensors. Therefore, RU amount in 1 mL of CS solution was varied between 5–20 mg by keeping TiO_2 (23.6 mg) and c-MWCNT (19.5 mg) amounts constant and four different DAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensors were fabricated. The response of these biosensors to histamine was recorded and the highest response was recorded with the biosensor fabricated with 20 mg RU (ESI, Fig.S2). Therefore, this amount was selected as the optimum amount for RU. Consequently, these optimum amounts of modification materials were used in the construction of the DAO/TiO_2–c-MWCNT–RU–CS/SPCE and MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensors. A series of biosensors containing different aliquots of DAO enzyme (2.5 µL; 5 µL; 7.5 µL and 10 µL) were prepared and the responses of these biosensors to histamine were investigated. DAO/TiO_2–c-MWCNT–RU–CS/SPCE response to histamine was relatively low at enzyme loading below 5 µL. The biosensor response increased from 2.5 to 5 µL where it showed an optimal response. The response then slightly decreased from 5 to 10 µL. In case of MAO the highest response was also obtained with 5 µL enzyme. Thus, 5 µL of DAO or MAO was used as the enzyme quantity for the fabrication of the biosensors (ESI, Fig. S3).

Surface Morphology

SEM images of (a) SPCE, (b) TiO_2–CS/SPCE, (c) c-MWCNT–CS/SPCE, (d) TiO_2-c-MWCNT–CS/SPCE, (e) RU–CS/SPCE, (f) TiO_2–c-MWCNT–RU–CS/SPCE, (g) DAO/TiO_2–c-MWCNT–RU–CS/SPCE and (h) MAO/TiO_2–c-MWCNT–RU–CS/SPCE were taken to investigate the morphologies of the modification materials (Fig. 1). Image a depicts the surface morphology of bare SPCE and image b shows that spherical nanoparticles of TiO_2 were homogenously dispersed on SPCE surface. Image c presents the SEM image of c-MWCNTs with a filamentous morphology. The SEM image of TiO_2-c-MWCNT–CS/SPCE (image d) shows that TiO_2 nanoparticles and c-MWCNTs were well dispersed in chitosan matrix without serious agglomeration. The large clustered structures in image e can be ascribed to the presence of RU on SPCE. In the SEM image of TiO_2–c-MWCNT–RU–CS/SPCE (image f) the typical filamentous morphology of the c-MWCNTs, globular structures of the TiO_2 nanoparticles and large clustered structures of RU can be easily observed. Moreover, the homogenous distribution of these materials on electrode surface and the porous morphology obtained provide a suitable platform for the immobilization of the enzymes. As shown in Fig. 1g when DAO was immobilized on TiO_2–c-MWCNT–RU–CS/SPCE, a slight change in morphology was observed. On the other hand, after the MAO enzyme was modified onto the TiO_2–c-MWCNT–RU–CS/SPCE the SEM image obviously changed indicating the successful immobilization of the enzyme onto electrode surface (Fig. 1h). The SEM image of

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MAO based electrode with lower magnification is presented in ESI Fig. S4.

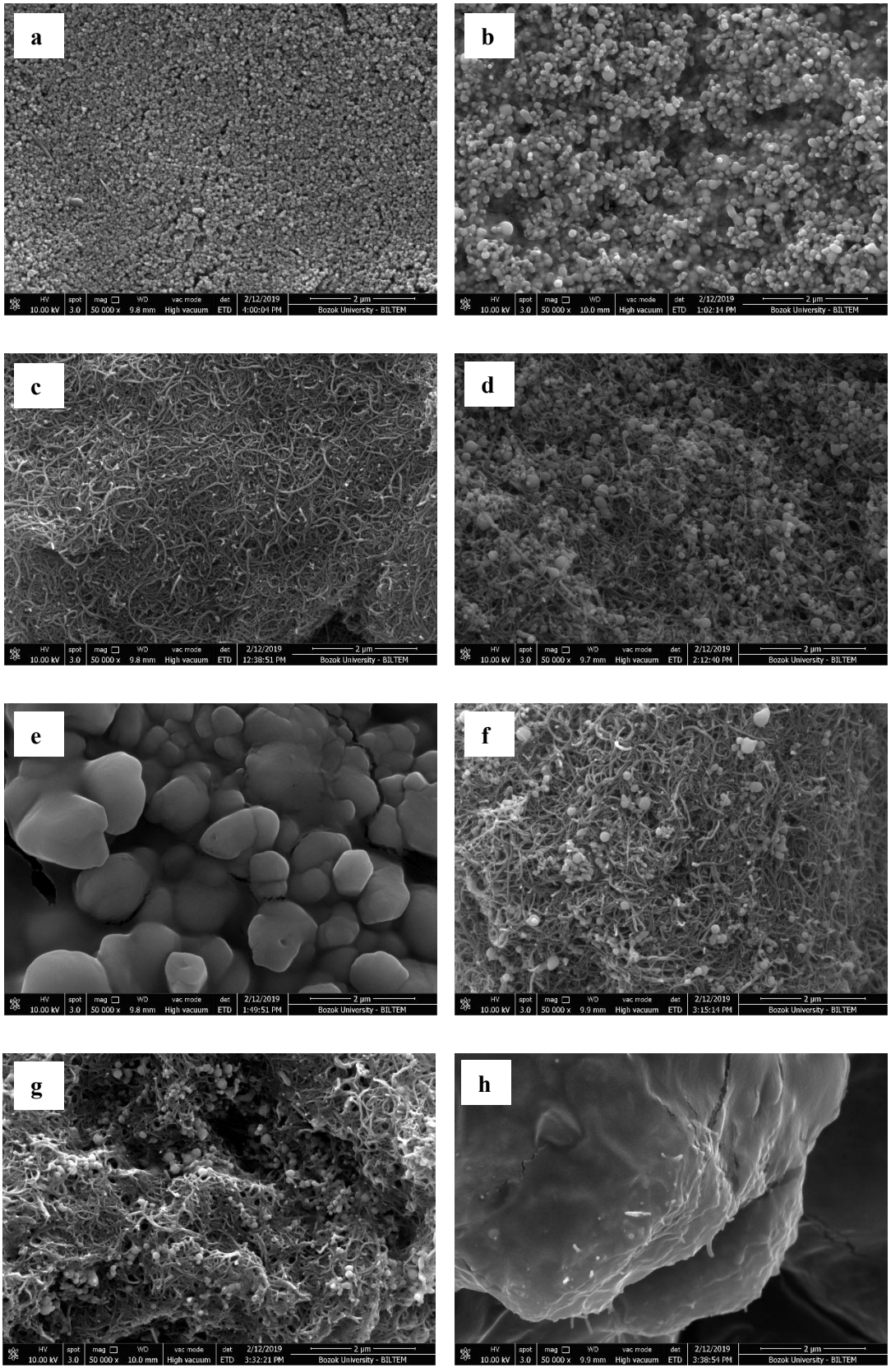

Fig. 1 SEM images of different modified surfaces: (a) SPCE, (b) TiO_2–CS/SPCE, (c) c-MWCNT–CS/SPCE, (d) TiO_2-c-MWCNT–CS/SPCE, (e) RU–CS/SPCE, (f) TiO_2-c-MWCNT-RU–CS/SPCE, (g) DAO/TiO_2-c-MWCNT-RU–CS/SPCE and (h) MAO/TiO_2-c-MWCNT-RU–CS/SPCE.

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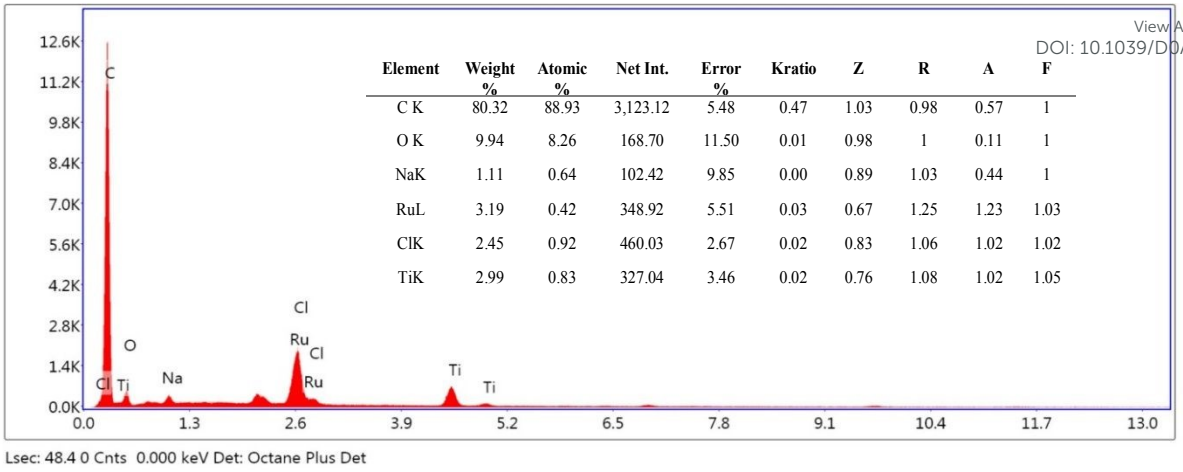

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	R	A	F
C K	80.32	88.93	3,123.12	5.48	0.47	1.03	0.98	0.57	1
O K	9.94	8.26	168.70	11.50	0.01	0.98	1	0.11	1
NaK	1.11	0.64	102.42	9.85	0.00	0.89	1.03	0.44	1
RuL	3.19	0.42	348.92	5.51	0.03	0.67	1.25	1.23	1.03
ClK	2.45	0.92	460.03	2.67	0.02	0.83	1.06	1.02	1.02
TiK	2.99	0.83	327.04	3.46	0.02	0.76	1.08	1.02	1.05

Fig. 2 EDX spectra and elemental analysis of TiO_2–c-MWCNT–RU–CS composite.

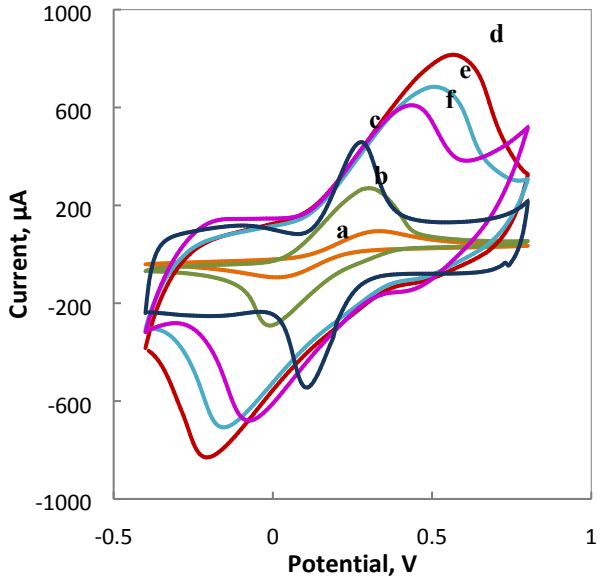

Fig. 3 Cyclic voltammograms of (a) SPCE, (b) TiO_2–CS/SPCE, (c) c-MWCNT–CS/SPCE, (d) TiO_2–c-MWCNT–RU–CS/SPCE, (e) DAO/TiO_2–c-MWCNT–RU–CS/SPCE and (f) MAO/TiO_2–c-MWCNT–RU–CS/SPCE in 5.0 mM $Fe(CN)_6^{4-/3-}$ containing 0.10 M KCl at a scan rate of 50 mV s^{-1}.

The EDX spectra of TiO_2–c-MWCNT–RU–CS composite is shown in Fig. 2. The C, O, Ru and Ti peaks obtained from EDX study verified the presence of TiO_2–c-MWCNT–RU–CS composite on the surface of the SPCE and strongly supported the SEM results of the corresponding electrode.

Electrochemical characterization

The electrochemical behavior of the modified electrodes was explored by CV using 5.0 mM $Fe(CN)_6^{4-/3-}$ containing 0.10 M KCl as a redox probe (Fig. 3). A couple of oxidation and reduction peaks corresponding to the redox behavior of $Fe(CN)_6^{4-/3-}$ were observed at bare SPCE (curve a). The modification of the bare SPCE with TiO_2 nanoparticles (curve b) or MWCNTs (curve c) resulted in higher peak currents indicating an increase in the effective surface. The highest peak currents were obtained with TiO_2–c-MWCNT–RU–CS/SPCE (curve d), which can be attributed to a further increase of effective surface area in the presence of c-MWCNTs, TiO_2 and RU. After DAO was immobilized onto TiO_2–c-MWCNT–RU–CS/SPCE (curve e), the peak currents slightly decreased because of enzyme hindering the transfer of electrons toward the electrode surface, which proved that DAO had been successfully immobilized on the TiO_2–c-MWCNT–RU–CS/SPCE[35]. Similar behavior was also observed with the MAO/TiO_2–c-MWCNT–RU–CS/SPCE (curve f).

Optimization of Experimental Parameters

Operating Potential

The operating potential has a great effect on biosensor performance since it contributes to its sensitivity and selectivity. Fig. 4 depicts the cyclic voltammograms of (a) TiO_2–c-MWCNT–RU–CS/SPCE, (b) RU–CS/SPCE and (c) SPCE taken in 0.05 M BR buffer solution containing 0.10 M KCl (pH 7.5) at a scan rate of 50 mVs^{-1}. As expected, no peaks are noticed in this potential range for bare SPCE (curve c). In the cyclic voltammograms of TiO_2–c-MWCNT–RU–CS/SPCE (curve a) and RU–CS/SPCE (curve b) an oxidation peak at about –0.35 V and a reduction peak at about –0.43 V can be observed. These peaks can be attributed to the electrochemical redox behavior of RU. Due to the CV results the response of DAO/TiO_2–c-MWCNT–RU–CS/SPCE to histamine was studied between −0.20 and –0.40 V to determine the optimum potential. The sensitivity increased as the potential becomes more negative from −0.20 to −0.40 V, and the highest sensitivity was obtained at –0.40 V (ESI, Fig. S5). However, the linear dynamic range and repeatability of the measurements were not satisfactory at –0.40 V. Therefore, an operating potential of –0.30 V was selected where a wider dynamic range and higher repeatability were obtained. H_2O_2 enzymatically generated from histamine in the presence of DAO or MAO (Equation 2) is reduced to H_2O by RU (II) ($Ru(NH_3)_6^{2+}$) and RU (III) ($Ru(NH_3)_6^{3+}$) is produced (Equation 3). RU (III) is then reduced to RU (II) (Equation 4) and the current response obtained is directly proportional to the

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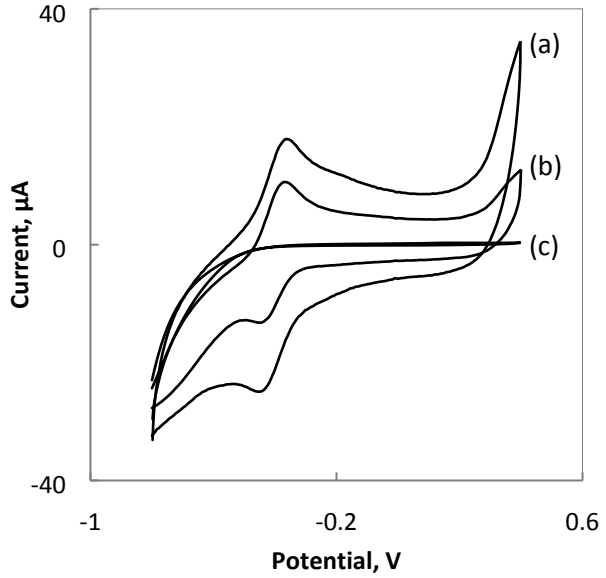

Fig. 4 Cyclic voltammograms of (a) TiO_2–c-MWCNT–RU–CS/SPCE, (b) RU–CS/SPCE and (c) SPCE (pH 7.5 0.05 M BR buffer solution in the presence of 0.10 M KCl, at 50 mVs^{-1} scan rate)

concentration of histamine[20]. The electrochemical reactions involved in response mechanism of the histamine biosensors are given below:

$RCH_2NH_2 + O_2 + H_2O \rightarrow RCHO + H_2O_2 + NH_3$ (in the presence of DAO or MAO) (2)

$2[Ru(NH_3)_6]^{2+} + H_2O_2 + 2H^+ \rightarrow 2[Ru(NH_3)_6]^{3+} + 2H_2O$ (3)

$[Ru(NH_3)_6]^{3+} + e^- \rightarrow [Ru(NH_3)_6]^{2+}$ (at −0.30 V) (4)

Buffer pH

The pH of the working buffer can influence the enzyme activity, resulting in the change of affinity of the enzyme to its substrate[36,37]. Therefore, the performance of the enzyme based sensors depends significantly on the pH of the buffer solution. The influence of buffer pH on the histamine responses of the DAO/TiO_2–c-MWCNT–RU–CS/SPCE and MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensors was investigated over the pH range between 6.0 and 9.0 in 0.05 M BR buffer. As can be seen from Fig. 5 the sensitivity of the DAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensor increased with the increase of pH from 6.0 to 7.5 and then decreased.

The highest sensitivity was obtained at pH 7.5 and this pH was selected as optimum pH. In case of MAO/TiO_2–c-MWCNT–RU–CS/SPCE, the optimum pH obtained was 9.0. DAO and MAO enzymes catalyze the oxidative deamination of various biogenic amines to the corresponding aldehydes and NH_3 with H_2O_2 production. So, the effect of buffer pH (0.05 M BR buffer solution) on the responses of DAO/TiO_2–c-MWCNT–RU–CS/SPCE and MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensors was also investigated for the other biogenic amines (cadaverine, putrescine, phenylethylamine, tryptamine, spermine and spermidine) widely present in food products. Both biosensors did not respond to tyramine, thus, pH optimization was not performed for this biogenic amine. Table 1 depicts the optimum pH values obtained for each biogenic amine with both biosensors. These optimum pH values were used for the determination of analytical characteristics of both biosensors for the corresponding biogenic amines.

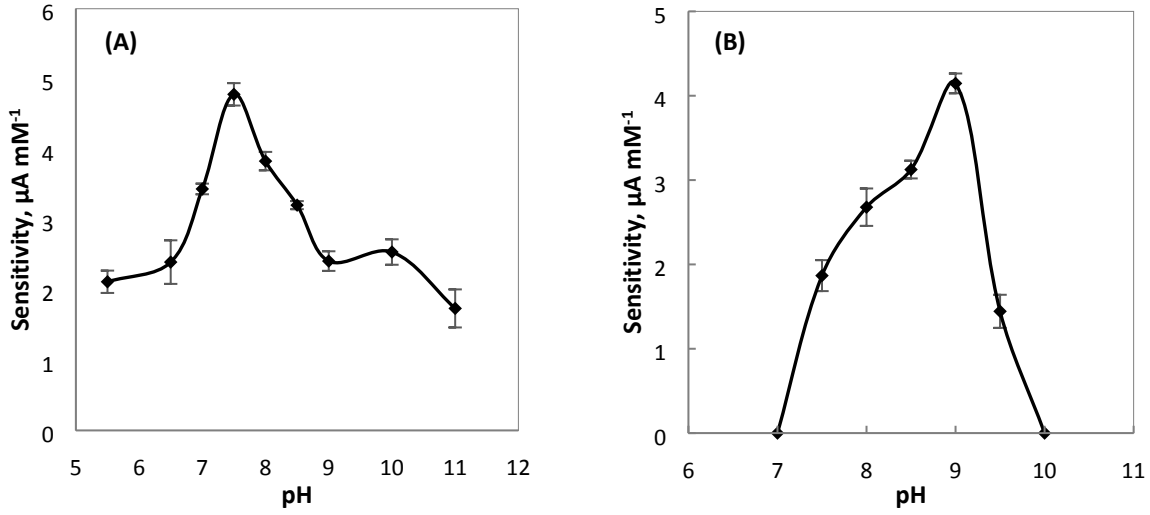

Fig. 5 Influence of pH on the histamine response of (A) DAO/TiO_2–c-MWCNT–RU–CS/SPCE and (B) MAO/TiO_2–c-MWCNT–RU–CS/SPCE (in 0.05 M BR buffer solution, E_{app} = − 0.30 V) Error bars show the standard deviation of three measurements.

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Table 1 Analytical performance characteristics for BAs obtained with DAO/TiO_2–c-MWCNT–RU–CS/SPCE and MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensors*

Substrate	DAO/TiO_2-c-MWCNT-RU-CS/SPCE			MAO/TiO_2-c-MWCNT-RU-CS/SPCE		
	pH	Linear working range, M	Sensitivity, μA/mM	pH	Linear working range, M	Sensitivity, μA/mM
Histamine	7.5	9.9×10^{-6} – 1.1×10^{-3}	3.39	9.0	5.6×10^{-5} – 1.1×10^{-3}	2.20
Tryptamine	10.0	1.4×10^{-4} – 1.3×10^{-3}	5.41	-	-	-
Putrescine	9.5	2.9×10^{-5} – 5.0×10^{-3}	0.74	9.0	9.9×10^{-6} – 8.2×10^{-4}	1.90
Cadaverine	6.0	9.1×10^{-5} – 6.2×10^{-3}	0.71	8.0	9.1×10^{-5} – 1.1×10^{-3}	1.02
Phenylethylamine	10.0	9.1×10^{-5} – 2.9×10^{-3}	2.38	9.0	2.2×10^{-4} – 2.9×10^{-3}	0.35
Spermine	8.5	5.7×10^{-5} – 3.5×10^{-4}	3.17	-	-	-
Spermidine	9.0	1.4×10^{-4} – 6.2×10^{-3}	0.66	-	-	-

*Chronoamperometric responses were measured at optimum pH of each amine

Analytical Performance Characteristics of the Biosensors

DAO and MAO are widely used as amine specific enzymes in the development of BA biosensors. However, a survey of the literature on BA biosensors shows that the substrate specificity of these enzymes is quite different[29, 38]. Moreover, parameters such as modification of the electrode or source of the enzymes may affect the substrate specificity of the enzymes. In this study the substrate specificity of DAO and MAO enzymes used for the development of TiO_2–c-MWCNT–RU–CS/SPCE based biosensors were compared. For this purpose, chronoamperometric responses of DAO/TiO_2–c-MWCNT–RU–CS/SPCE and MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensors to histamine, cadaverine, putrescine, phenylethylamine, tryptamine, spermine and spermidine were investigated and *i-t* graphs were recorded for each biogenic amine at its optimum pH. The calculated current differences (Δ*I*) were plotted against biogenic amine concentration (ESI, Fig. S6 and S7). The linear working ranges and sensitivities obtained from corresponding calibration graphs are presented in Table 1. As seen from the Table both biosensors responded to histamine with a wide linear working range and good sensitivity.

The *i-t* graphs and calibration curves obtained for histamine with DAO/TiO_2–c-MWCNT–RU–CS/SPCE and MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensors are given in Fig. 6. DAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensor displayed a linear relationship between the current and histamine concentration in the range of 9.9×10^{-6}–1.1×10^{-3} M with a sensitivity of 3.39 μA mM^{-1}. The detection limit of the biosensor was calculated according to the $3s_b/m$ criteria where *m* represents the slope of the calibration graph and s_b represents the standard deviation of the chronoamperometric responses from different solutions of histamine at the concentration level corresponding to the lowest concentration of the calibration plot[39]. The detection limit of the DAO based biosensor was found to be 6.9×10^{-6} M. For the MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensor the linear dynamic range of the histamine determination was from 5.6×10^{-5}–1.1×10^{-3} M. The detection limit and sensitivity of this biosensor were found to be 3.6×10^{-5} M and 2.20 μA mM^{-1}, respectively. Both biosensors exhibited a rapid electrochemical response, reaching 95% of the steady-state current within 30 s after each histamine addition.

DAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensor exhibited the best sensitivity to tryptamine (5.41 μA mM^{-1}). However, the linear dynamic range obtained for tryptamine was very narrow (1.4×10^{-4} – 1.3×10^{-3} M). The linear ranges of other BAs were from 5.7×10^{-5} to 3.5×10^{-4} for spermine (3.17 μA mM^{-1}), from 1.4×10^{-4} to 6.2×10^{-3} for spermidine (0.66 μA mM^{-1}), from 2.9×10^{-5} to 5.0×10^{-3} M for putrescine (0.74 μA mM^{-1}) and from 9.1×10^{-5} to 6.2×10^{-3} M for cadaverine (0.71 μA mM^{-1}). Phenylethylamine could be detected in the range of 9.1×10^{-5} – 2.9×10^{-3} M (2.38 μA mM^{-1}). It can be concluded that DAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensor can be also used in the determination of cadaverine, putrescine, phenylethylamine, spermine and spermidine within a wide linear range.

On the other hand, MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensor did not respond to tryptamine, tyramine, spermine and spermidine. This biosensor exhibited linear response to putrescine from 9.9×10^{-6} to 8.2×10^{-4} M with a sensitivity of 1.90 μA mM^{-1} which was similar to that obtained for histamine (2.20 μA mM^{-1}). In case of cadaverine and phenylethylamine the linear ranges were between 9.1×10^{-5} – 1.1×10^{-3} M (1.02 μA mM^{-1}) and 2.2×10^{-4} – 2.9×10^{-3} M (0.35 μA mM^{-1}), respectively. These results indicated that both biosensors can be used in the determination of the most important amines, histamine, putrescine and cadaverine.

Table 2 presents the analytical performance characteristics of various electrochemical biosensors previously reported for the determination of histamine. It can be seen from the Table that DAO/TiO_2–c-MWCNT–RU–CS/SPCE and MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensors offered a wider linear dynamic range for histamine than most of the previous histamine biosensors.

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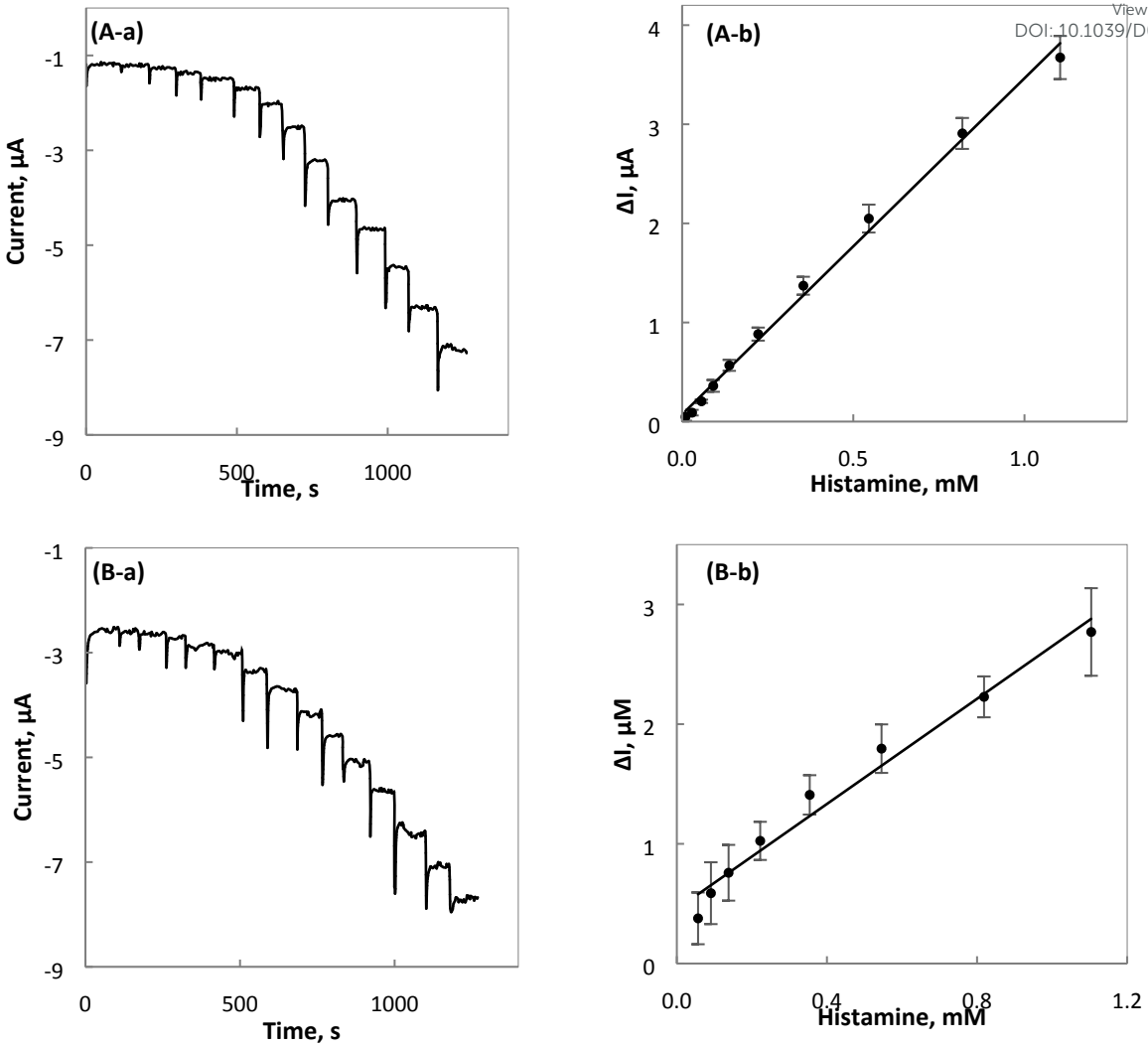

Fig. 6 (a) Chronoamperometric *i-t* graphs and (b) calibration curves of (A) DAO/TiO_2–c-MWCNT–RU–CS/SPCE (pH 7.5) and (B) MAO/TiO_2–c-MWCNT–RU–CS/SPCE (pH 9.0) for histamine in 0.05 M BR buffer at −0.30 V. The error bars are calculated from measurements performed with three different biosensors.

Moreover, the sensitivities obtained with the presented biosensors were higher than some of the listed works. These results suggested that DAO or MAO immobilized TiO_2–c-MWCNT–RU–CS/SPCE is a suitable platform for histamine determination.

Reusability and Reproducibility

The reusability and reproducibility of the DAO/TiO_2–c-MWCNT–RU–CS/SPCE and MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensors were explored using histamine as substrate. Five consecutive calibration curves were performed under the optimized operational conditions using the same biosensor to investigate the reusability. The decrease observed in sensitivity (slope of the linear calibration) in terms of relative standard deviation (RSD) was <5% between the 1st and the 5th calibration with both biosensors. This result indicated the high reusability of the presented biosensors and their potential application in continuous monitoring systems such as flow injection analysis. The high reusability obtained can be attributed to the good interaction between DAO or MAO enzymes and TiO_2–c-MWCNT–RU–CS matrix. Moreover, the optimization of TiO_2 and c-MWCNT amounts by CDD resulted in a thick modification layer on electrode surface which might decrease the leaching of enzyme from the electrode surface. In case of reproducibility, five consecutive calibration curves were plotted, using five different similarly fabricated electrodes. The RSD values obtained with DAO/TiO_2–c-MWCNT–RU–CS/SPCE and MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensors were 3.9% and 4.3%, respectively. It can be concluded that the biosensors exhibited good reproducibility.

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Table 2 Analytical performance characteristics of various electrochemical biosensors previously reported for the determination of histamine

No	Electrode modification	Working potential (V)/pH	Limit of Detection	Linear Dynamic Range	Sensitivity	Real Sample	References
1	DAO/CeO_2–PANI/GCE	/7.4	48.7 µM	450–1050 µM	51.22 µA mM^{-1}	Fish muscle	14
2	MWCNTs/p-(AHNSA)/GCE	/7.0	0.0762 µM	0.1–100 µM	776 µA mM^{-1}	Fish muscle	40
3	DAO-nPt/GPH/Chitosan/CSPE	+0.40/7.4	0.0254 µM	0.1 µM –300 µM	63.1 µA mM^{-1}	Fish	15
4	DAO/BSA/GA/SPCE	-0.30/7.2	4.5 µM	9 – 900 µM	0.422 µA mM^{-1}	Fish	41
5	DAO-HEMA film/Carbon paste SPE	+0.35/7.4	5.8 µM	0–326 µM	1.02 µA mM^{-1}	Tiger prawn	6
6	HO/HRP-CPE	-0.02/7.5	0.069 µM	Up to 0.95 µM	1.2 µA mM^{-1}	Fish	42
7	AO-HRP-PVI_{13}-dmeOs-PEGDGE/Graphite electrode	-0.05/7.2	0.3 µM	1–150 µM	5.33 µA mM^{-1}	Fish	43
8	DAO/TiO_2–c-MWCNT–RU–CS/SPCE	-0.30/7.5	6.9 µM	9.9–1100 µM	3.39 µA mM^{-1}	Fish	This work
9	MAO/TiO_2–c-MWCNT–RU–CS/SPCE	-0.30/9.0	36 µM	56–1100 µM	2.20 µA mM^{-1}	Fish	This work

AO: amine oxidase; PVI_{13}-dmeOs: BSA: bovine serum albumine; CPE: carbon paste electrode; CSPE: carbon screen printed electrode; HEMA: 2-hydroxyethyl methacrylate; GA: glutaraldehyde; GPH: reduced graphene oxide; GCE: glassy carbon electrode; HO: histamine oxidase; HRP: horseradish peroxidase; nPt: platinum nanoparticles; PANI: polyaniline; PEGDGE: poly(ethylene glycol) (400) diglycidyl ether; PVI_{13}-dmeOs: poly(1-vinylimidazole)-[Os(4,4′-dimethylbipyridine)$_2$Cl]$^{+/2+}$ complex; PS: polysulfone; p-(AHNSA): poly(4-amino-3-hydroxynaphthalene sulfonic acid), SPE: screen printed electrode

Interference Effect

The possible interference from various amino acids (histidine, ornithine, tyrosine, lysine, tryptophan, arginine, and phenylalanine) was investigated. The amino acids studied were those involved in the biosynthesis of BAs[4]. The interference effect was calculated by comparison of the response obtained for 0.04 M standard histamine solution vs. the response obtained for solutions of the same concentration of those amino acids. Fig. 7 indicates that ornithine, arginine, tryptophan and phenylalanine have a slightly effect (<5%) on the response of DAO/TiO_2–c-MWCNT–RU–CS/SPCE. However, histidine (18.2%), lysine (24.5%) and tyrosine (20.2%) cause significant interference on the response current of DAO/TiO_2-c-MWCNT-RU–CS/SPCE. In case of MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensor ornithine and tyrosine did not interfere with the histamine determination causing less than <1% change in biosensor response. On the other hand, the presence of equal concentration of histidine (15.5%), lysine, (29.5%), arginine (44.7%), tryptophan (12.8%)

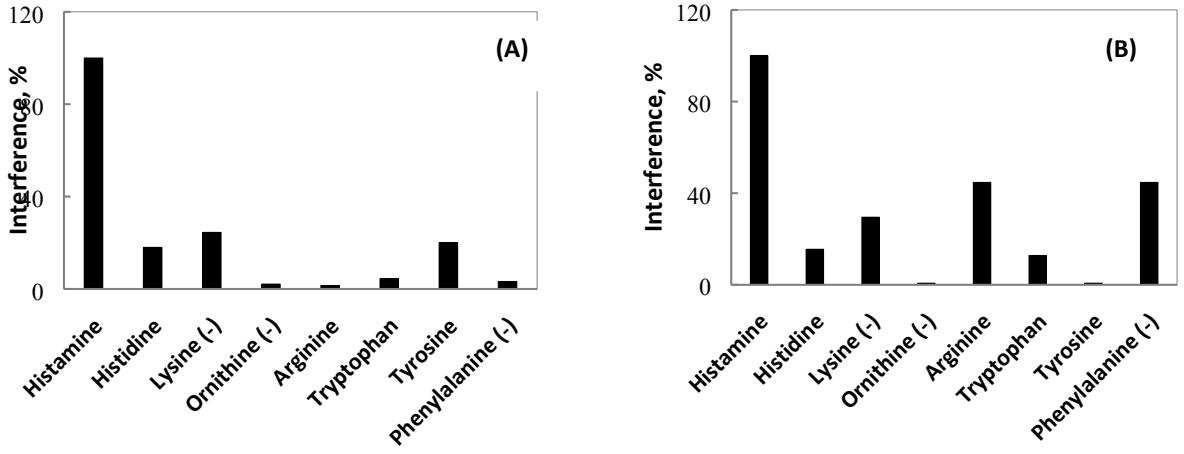

Fig. 7 Effect of potentially interfering amino acids on the response of (A) DAO/TiO_2–c-MWCNT–RU–CS/SPCE and (B) MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensors (in 0.05 M BR buffer solution, E_{app} = − 0.30 V*)*

and phenylalanine (44.7%) cause higher chronoamperometric responses of the histamine determination and their existence in real samples can affect the histamine response of the biosensor. In real sample analysis standard addition method can be used to eliminate the effect of these interfering substances.

Application in Fish Sample

The practical application of the developed biosensors was evaluated by means of the histamine analysis in fish sample. Histamine level in fish sample was determined by standard addition method. For this purpose, additions of standard histamine solution were made to the fish extract, and a standard addition calibration curve was obtained. The histamine concentration in fish sample was calculated from this curve and found to be $8.957(\pm0.266)\times10^{-2}$ mM (n=3) with DAO/TiO_2–c-MWCNT–RU–CS/SPCE and $8.428(\pm0.294)\times10^{-2}$ mM (n=3) with MAO/TiO_2–c-MWCNT–RU–CS/SPCE. Recovery tests in fish sample were also performed to provide further support for the applicability of the method. For this purpose, fish extract was spiked with 2.0×10^{-2} mM and 4.0×10^{-2} mM of standard histamine solution and total histamine levels in spiked fish samples were determined by standard addition in triplicate. Table 3 presents the recoveries of histamine from spiked fish sample determined by the DAO/TiO_2–c-MWCNT–RU–CS/SPCE and MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensors. The average recoveries obtained for histamine were varied in the range from 100.0 to 104.6%, indicating that the reliability of the proposed biosensor method is quite good.

Conclusion

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In this study, novel biosensors based on SPCEs modified with c-MWCNTs, TiO_2 nanoparticles, RU and DAO or MAO are reported for the determination of histamine. The composite use of the c-MWCNTs, TiO_2 nanoparticles and RU improved the analytical performance of the presented DAO/TiO_2–c-MWCNT–RU–CS/SPCE and MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensors and they exhibited, wide linear dynamic range, low detection limit, good repeatability and reproducibility towards histamine determination. The DAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensor responded to all BAs investigated, however the MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensor showed no response towards tryptamine, spermine and spermidine. The presented biosensors were successfully used as efficient monitoring tool to determine histamine in fish sample.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Table 3 The result of the recovery studies of standard additions to fish samples obtained with DAO/TiO_2–c-MWCNT–RU–CS/SPCE and MAO/TiO_2–c-MWCNT–RU–CS/SPCE biosensors

DAO/TiO_2–c-MWCNT–RU–CS/SPCE			MAO/TiO_2–c-MWCNT–RU–CS/SPCE		
Histamine added, mM	**Histamine found, mM[a]**	**Recovery, %**	**Histamine added, mM**	**Histamine found, mM[a]**	**Recovery, %**
-	$8.957(\pm0.266)\times10^{-2}$	-	-	$8.428(\pm0.294)\times10^{-2}$	-
2.0×10^{-2}	$10.972(\pm0.003)\times10^{-2}$	101.1(±0.2)	2.0×10^{-2}	$10.420(\pm0.009)\times10^{-2}$	100.0(±0.5)
4.0×10^{-2}	$13.118(\pm0.042)\times10^{-2}$	104.6(±1.1)	4.0×10^{-2}	$12.457(\pm0.034)\times10^{-2}$	101.3(±0.8)

[a] Results are the mean value of three measurements

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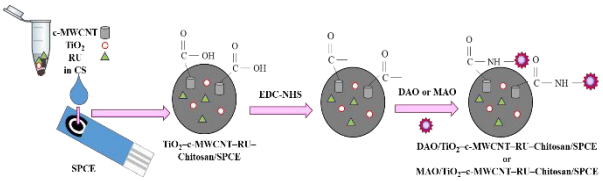

Disposable biosensors based on carboxylated carbon nanotubes, titanium dioxide nanoparticles, hexaammineruthenium chloride and diamine oxidase or monoamine oxidase modified screen printed electrodes were developed for rapid and reliable determination of histamine in fish sample.