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Diamine oxidase-modified screen-printed electrode for the redox-mediated determination of histamine



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Abstract

Histamine is an important biogenic amine because of its role in immune responses and the regulation of physiological functions. It is also used as a food freshness indicator, so its maximum concentration in fish is legally regulated. Although several robust and sensitive methods for histamine detection are already available, it continues to be a challenge to develop simple and portable devices that allow rapid histamine screening at any point of the fish production chain. Thus, in this work, a simple, miniaturized and low-cost sensor for histamine analysis was developed. The construction of the sensor only takes 30 min and consists of the immobilization of the enzyme diamine oxidase on the surface of a screen-printed carbon electrode by cross-linking. The quantification of histamine was achieved by chronoamperometry (+ 0.2V, 120 s) using hexacyanoferrate (III) as a redox mediator. This selective sensor provided a low limit of detection (0.97 mg L⁻¹) and accurate and precise results and was successfully applied to the analysis of spiked tuna and mackerel extracts, obtaining recovery values of 99–100%. Moreover, the sensor shows good stability, maintaining 87.7% of its initial signal after 35 days.

Keywords: Amperometric biosensor, Enzymatic sensor, Biogenic amine, Histamine, Diamine oxidase, Redox mediator, Ferrocyanide, Screen-printed electrode

Introduction

Histamine is a low molecular weight biogenic amine produced by microbial decarboxylation of the amino acid histidine. It can be found in various kinds of foods such as meat, fish, and fermented foods (Biji et al. 2016; Papageorgiou et al. 2018). Although the low levels of histamine normally present in food are not considered a health risk, the intake of high amounts of histamine causes scombroid poisoning. This is also known as histamine fish poisoning since it is mostly related to the consumption of spoiled fish. This food poisoning continues to be a common cause of food intoxication worldwide and produces both physiological and toxicological effects (e.g., abdominal pain, diarrhea, vomiting, headache, urticaria...), the severity of which depends on the dosage (Feng et al. 2016; Hungerford 2010). The rapid increase of histamine levels in fish is mainly caused by the presence of large amounts of histidine and inappropriate storage conditions after capture (temperatures > $4\,^{\circ}\mathrm{C}$ and/or long storage times) that promote bacterial activity (Kung et al. 2015; Silva et al. 1998; Tsai et al. 2005). Thus, in histidine-rich fish species, e.g., sardine, tuna, and mackerel, it is more likely that histamine is developed than in histidine-poor species such as hake. Moreover, histamine is not affected by freezing, heating, sterilization, or canning processes (Naila et al. 2010; Visciano et al. 2014). Hence, histamine is a reliable fish freshness indicator and its concentration is legally regulated: EU legislation establishes 200 mg kg $^{-1}$ (for individual samples) and 100 mg kg $^{-1}$ (as average) as limits for histidine-rich fish species (Off. J. Eur. Union. Comm. Regul. No 2073/2005 15 November 2005).

Therefore, histamine analysis is key not only to assure food freshness and quality, but also to avoid food intoxications. Analytical methods typically used for histamine analysis include separation techniques, e.g., chromatography (gas and liquid) or capillary electrophoresis, coupled to UV-Vis, fluorescence, or mass spectrometric

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detection (Almeida et al. 2012; Daniel et al. 2015; Evangelista et al. 2016; Nadeem et al. 2019; Nei et al. 2017; Papageorgiou et al. 2018). These methods are very robust and selective; however, they have drawbacks since they often require (1) long analysis times, (2) expensive and complex instrumentation (which need expensive maintenance and highly qualified operators), (3) tedious and time-consuming sample treatments (e.g., digestion and derivatization), and (5) large amounts of reagents and solvents, producing large amounts of waste. Moreover, the equipment is not portable; hence, on-site analysis is not possible.

Considering that the concentration of histamine undergoes a rapid increase when the storage conditions of the fish are not adequate (Kung et al. 2015; Silva et al. 1998; Tsai et al. 2005), the development of portable devices that allow the monitoring of its levels from fishing to the final consumer is of high interest. Electrochemical biosensors are interesting tools since they offer fast analysis and accurate results and show an excellent ability for miniaturization, providing portable platforms that allow decentralized analysis. Due to the increasing demand for fast and reliable information, the development of portable sensors is a continuously growing area. Furthermore, with the advances in smartphone technology and digital communication networks, sensors integrated with electronics are becoming essential tools in daily life (Dincer et al. 2019; Quesada-González and Merkoçi 2017; Zarei 2017).

In this context, several works reported electrochemical devices for histamine analysis (Ordóñez et al. 2016; Papageorgiou et al. 2018). Some of them were based on the electrochemical oxidation of histamine on modified electrodes such as carbon paste electrodes modified with carbon nanotubes (Stojanović et al. 2016) or rhenium oxides (Veseli et al. 2016), nafion-coated copper phosphate nanostructured screen-printed carbon electrodes (Lee et al. 2018), and glassy carbon electrodes modified with polystyrene-graphene oxide (Saghatforoush et al. 2014) or a carbon nanotube-coated polymer (Geto et al. 2014). These sensors usually require the pre-treatment of the electrodes and the use of metals, polymers, and/or nanomaterials, which increases the cost and construction time. Sensors based on electrodes modified with enzymes (Apetrei and Apetrei 2016; Gumpu et al. 2014; Pérez et al. 2013; Zeng et al. 2000), molecularly imprinted polymers (Akhoundian et al. 2017; Basozabal et al. 2014) and antibodies (Dong et al. 2017) are usually more selective due to the incorporation of a recognition element. However, the immobilization of these elements is often a complex and time-consuming task (Akhoundian et al. 2017; Apetrei and Apetrei 2016; Basozabal et al. 2014; Dong et al. 2017; Gumpu et al. 2014; Pérez et al. 2013; Zeng et al. 2000).

The biosensor developed in this work stands out for its simplicity without sacrificing analytical performance. Screen-printed electrodes were used to obtain a miniaturized, portable and robust sensor that allows analysis of a small sample volume (30–40 μ L). This sensor is a step forward regarding our previously developed histamine sensor (Torre et al. 2019) in which the enzyme diamine oxidase (DOX) was immobilized by cross-linking using glutaraldehyde and bovine serum albumin. In this work only glutaraldehyde was used, simplifying the sensor's construction and achieving better stability. On the other hand, the use of a redox mediator allowed histamine analysis through its electrochemical oxidation, improving the sensitivity (more than 100-fold) and selectivity of the sensor's response, and avoiding matrix effects of the fish samples. Thus, a very simple sensor was obtained that not only showed good analytical features (in terms of limit of detection, sensitivity, reproducibility, and stability), but was also cheap, small-sized, and able to perform on-site analysis using low amounts of reagents and samples.

Methods/experimental

Solutions and equipment

Diamine oxidase (DOX, 0.11 U mg⁻¹), potassium hexacyanoferrate (III), biogenic amines (histamine, putrescine, spermine, tyramine, tryptamine, and phenylethylamine), and glutaraldehyde (GA) were supplied by Sigma-Aldrich. The fresh tuna and mackerel were purchased in a local market.

Working solutions of DOX, GA, histamine, and the other biogenic amines were prepared in 0.1 M phosphate buffer (PB) pH 7.2. This pH was chosen because the supplier indicated that this is the optimum pH to dissolve the enzyme and because the ideal pH range is 6.3–7.4 when histamine is used as the enzymatic substrate.

Type I deionized water (resistivity = $18.2\,M\Omega$ cm) was used throughout the work. All the chemicals were of analytical reagent grade and were used without further treatment or purification.

A Metrohm-Autolab potentiostat/galvanostat (PGSTAT 101) controlled by NOVA software (v1.10) was used for the electrochemical measurements. Screen-printed electrodes (printed on a ceramic substrate – $3.4~\rm cm \times 1.0~\rm cm$), consisting of a circular-shaped carbon-ink working electrode (WE, d = 4 mm), a silver-ink pseudoreference electrode (RE), and a carbon-ink counter electrode (CE) were used. These screen-printed carbon electrodes (SPCEs) and the suitable connector were purchased from DropSens.

Enzyme immobilization

The optimized procedure for the immobilization of the enzyme consisted of depositing $2\,\mu L$ of a 0.50-mg μL^{-1} DOX solution and $1\,\mu L$ of a 0.50% GA solution on the

WE of the SPCE, which was left to dry for 30 min at room temperature. The biosensor was ready to use after a washing step with buffer solution (0.1 M PB pH 7.2).

Electrochemical analysis

For the measurements, $20~\mu L$ of a standard/sample solution and $20~\mu L$ of a potassium hexacyanoferrate (III) solution (5 mM in the optimized procedure) were deposited on the SPCE. After 5 min, to allow the enzymatic reaction to occur, chronoamperograms were recorded at +0.2~V for 120~s, assuring that the current plateau was reached. The signal for each histamine concentration was the average of the currents obtained for the last 5~s of the chronoamperogram.

Fish samples preparation

Histamine was extracted from the fish samples (tuna and mackerel) following the same procedure as for the commercial R-Biopharm enzymatic test (R-Biopharm AG, n.d). This protocol was as follows: 20 mL of deionized water was added to 5.0 g of fish and mixed with a vortex mixer. This mixture was placed in a boiling water bath for 20 min and then centrifuged for 2 min at 10, 000g. Finally, the supernatant was removed and stored at $-80\,^{\circ}\text{C}$.

Results and discussion

In the presence of oxygen, DOX catalyzes the oxidation of histamine to the corresponding aldehyde (imidazole acetaldehyde), hydrogen peroxide, and ammonia (Fig. 1). In

work, the mediator hexanocyanoferrate (III) $([Fe(CN)_6]^{3-})$ (i.e., the oxidized form of the mediator) was used to record the electrochemical signal. An increase of the current was observed at + 0.2 V when the concentration of histamine increased. This current increase was due to the oxidation of the mediator (that showed an anodic peak at +0.12 V when a DOX/GA-modified electrode SPCE was used; Additional file 1: Figure S1). To be able to measure the electrochemical oxidation of the mediator, it must be previously reduced. This reduction could occur through the oxidation of H2O2 (produced during the enzymatic oxidation of histamine; Fig. 1) (Leonardo and Campàs 2016), which is common for other enzymatic sensors (Chaubey and Malhotra 2002; Gorton 1995). However, Keow et al. (Keow et al. 2012; Keow et al. 2007) showed that imidazole acetaldehyde produced during the enzymatic oxidation of histamine (Fig. 1) is oxidized at + 0.35 V (using a carbon-paste screen-printed electrode vs. an Ag/AgCl referenceelectrode). Since H₂O₂ is oxidized at potentials higher than + 0.6 V (Chaubey and Malhotra 2002; Gorton 1995) and imidazole acetaldehyde is oxidized at +0.35 V, the predominant mechanism should be the one in which the mediator is reduced by the oxidation of imidazole acetaldehyde (indicated in Fig. 1). Therefore, histamine was detected indirectly through the electrochemical oxidation of the enzymatically generated hexacyanoferrate (II) $([Fe(CN)_6]^{4-})$. The current intensity was measured by chronoamperometry, which is a simple detection technique that is very suitable for portable devices.

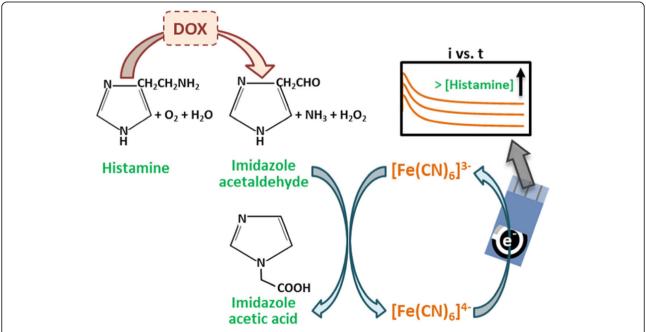


Fig. 1 Schematic representation of the proposed mechanism for the enzymatic and electrochemical reactions involved in the detection of histamine

Optimization studies

To determine the most suitable chronoamperometric measuring potential, a cyclic voltammogram (CV) of a 5.0-mM hexacyanoferrate (II) solution (in 0.1 M PB pH 7.2) using the sensor (DOX/GA/SPCE) was recorded. As can be seen in Additional file 1: Figure S1, an anodic peak at +0.12 V and a cathodic peak at -0.03 V were obtained. To guarantee the oxidation of the mediator, potentials starting at +0.2 V were considered to record the chronoamperograms. A study to determine the best measuring potential was performed by recording chronoamperograms using histamine solutions of 25 and 75 $mg L^{-1}$ at + 0.20 V and + 0.25 V. Similar analytical signals $(\Delta i$, difference between i for histamine and i for blank) were obtained for both potentials (Fig. 2). Thus, + 0.20 V was chosen as the detection potential since this potential lowers the possibility of interference of other electroactive species that can be oxidized at higher potentials.

The immobilization of the enzyme on the working electrode is also crucial for the adequate performance of the sensor. A saturated enzyme solution (0.50 mg μL^{-1}) was used. The effect of the GA concentration on the analytical signal was tested (Fig. 2). A GA concentration of 0.50% showed the highest Δi for both 25 and 75 mg L^{-1} histamine concentrations. This can be explained by a weak enzyme immobilization on the WE when a lower GA concentration (0.25%) is used, and a blocking effect of the electrode surface when a higher GA concentration (0.75%) is used. Moreover, 0.50% GA provided the most precise results. Therefore, this GA concentration was chosen as an optimum concentration to develop the sensor.

Finally, the concentration of the mediator was also optimized. As can been seen in Fig. 2, a 5.0-mM hexacyanoferrate (III) solution provided a higher analytical signal for $75~{\rm mg\,L^{-1}}$ of histamine than a 2.5-mM solution. On the other hand, the precision for both histamine concentrations is better for a 5.0-mM hexacyanoferrate (III) solution than for a 7.5-mM solution. Therefore, 5.0 mM of hexacyanoferrate (III) was chosen for the subsequent studies.

Analytical features of the sensor

To establish the analytical features of the developed sensor, chronoamperograms for different histamine concentrations were recorded (Additional file 1: Figure S2). A linear relationship between Δi and the histamine concentration was found in the concentration range between 5.0 and 75.0 mg L⁻¹ with a sensitivity of 38.9 nA L mg⁻¹ cm⁻² and a correlation coefficient of 0.99991 (Fig. 3a). The limit of detection (LOD), calculated as $3S_b/m$, and the limit of quantification (LOQ), calculated as $10S_b/m$ (where S_b is the standard deviation of the intercept and m is the slope of the calibration plot) were 0.97 and 3.2 mg L⁻¹, respectively. Additional file 1: Table S1 summarizes these and other figures of merit. This sensor exhibits high precision as demonstrated by the coefficient of variation, which is below 1.8%. In order to assess the reproducibility of the results, five equally prepared sensors were used to measure 10-mg L⁻¹ and 50-mg L⁻¹ histamine solutions, obtaining RSD values of 3.4% and 3.1%, respectively. When compared with previously reported electrochemical sensors for histamine detection, the developed sensor shows a comparable, or in some cases better, linear range, limit of detection, and/or precision (Table 1). This is more

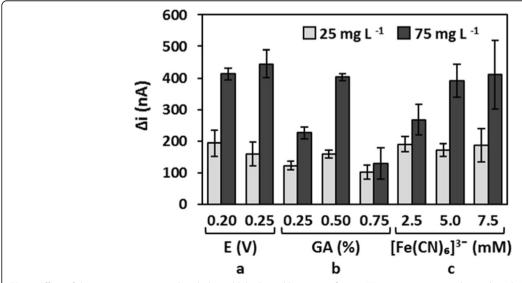


Fig. 2 Effect of the measuring potential and glutaraldehyde and hexacyanoferrate (III) concentration on the analytical signal (Δ i) for 25 and 75 mg L⁻¹ histamine. Experimental conditions: (**a**) DOX/GA/[Fe(CN)₆]³⁻, 0.50 mg μL⁻¹/0.50%/5.0 mM; (**b**) DOX/[Fe(CN)₆]³⁻, 0.50 mg μL⁻¹/5.0 mM; and (**c**) DOX/GA, 0.50 mg μL⁻¹/0.50%. Average values \pm *SD* obtained with three different sensors are represented

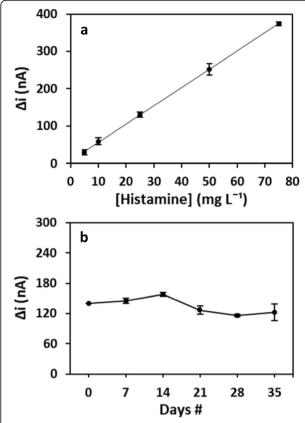


Fig. 3 a Representation of Δi vs. different histamine concentrations (5, 10, 25, 50, and 75 mg L⁻¹) obtained with the developed sensor by chronoamperometry at +0.20 V for 120 s. **b**) Δi for 25 mg L⁻¹ histamine obtained with the same sensor along 35 days. Average values \pm *SD* obtained with three different sensors are represented

valuable considering its simplicity and the short construction time (just 30 min vs. 1.0 - 68 h necessary for other sensors).

Stability

To assess the stability of the sensor's response, three sensors were used to analyze 25-mg $\rm L^{-1}$ histamine solutions on the day of their construction and after 7, 14, 21, 28, and 35 days. During this time, the sensors were stored at 4°C and protected from light. As can be seen in Fig. 3b, after 35 days, the sensor maintained 87.7% of the initial analytical signal, showing storage stability comparable with other histamine sensors (see Table 1). Therefore, the developed sensor showed good stability that demonstrates the adequate immobilization of the enzyme while maintaining its bioactivity.

Selectivity/interference studies

The selectivity of the sensor towards histamine was evaluated by analyzing other biogenic amines that could be present in fish. Thus, the biosensor's response towards phenethylamine, spermine, tyramine, tryptamine, and putrescine was assessed by comparing the signal (current intensity) obtained for a 25-mg L^{-1} solution of histamine vs. the signal for 25-mg L^{-1} solutions of each biogenic amine. As shown in Fig. 4, the signal for all the other biogenic amines was similar to the signal obtained for the blank, confirming the selectivity of the proposed sensor. Moreover, when a mixture of biogenic amines (histamine + the other biogenic amines) was analyzed, the signal obtained was nearly the same as the one obtained for a 25-mg L^{-1} histamine solution (417 nA vs. 451 nA, a

Table 1 Analytical characteristics of previously reported electrochemical devices for histamine

Electrode	Construction	Detection	Construction time (h)	Linear range (mg L ⁻¹)	LOD (mg L ⁻¹)	RSD (%)	Storage stability	Ref.
SPCE	DOX/GA/[Fe(CN) ₆] ³⁻	Amperometry	≈ 0.5	5–75	0.97	3.4	87.7%, 35 days	This work
SPCE (as WE)	DOX/PhotoHEMA	Amperometry	n.r.	5–60	0.65	7.6		(Keow et al. 2012)
SPCE	DOX/PhotoHEMA		n.r.	20-100	2.46	n.r.	n.r.	
SPCE	[Fe(CN) ₆] ⁴⁻ /DOX/ PhotoHEMA		n.r.	10–80	n.r.	n.r.		
SPCE	DOX/PhotoHEMA	Amperometry	n.r.	5-60	0.65	4.87	n.r.	(Keow et al. 2007)
SPCE	Nafion/Cu ₃ (PO ₄) ₂	Amperometry	≈ 1	5-100	3	7.2	n.r.	(Lee et al. 2018)
SPCE	Rhenium(IV) oxide	Amperometry	≈ 13	0.5-10	0.2	11	n.r.	(Veseli et al. 2016)
SPCE	DOX/HRP/polysulfone/ CNTs/ferrocene membrane	Amperometry	≈ 1.5	0.033 – 2.22	0.018	5.6-6.5	≈ 90%, 30 days	(Pérez et al. 2013)
ISE	MIP-NPs membrane	Potentiometry	> 48	0.11 – 1111.5	0.12	n.r.	≈ 100%, 10 days	(Basozabal et al. 2014)
GCE	Thin film of mercury	Potentiometry	≈ 0.5	1.5 – 10.5; 5–30; 20–90	1.31	2.14– 2.99	n.r.	(Švarc-Gajić and Stojanović 2011)
GCE	Ceria-polyaniline NPs/ DOX/nafion	CV	> 68	50–116.7	5.4	0.05- 0.10	86%, 18 days	(Gumpu et al. 2014)

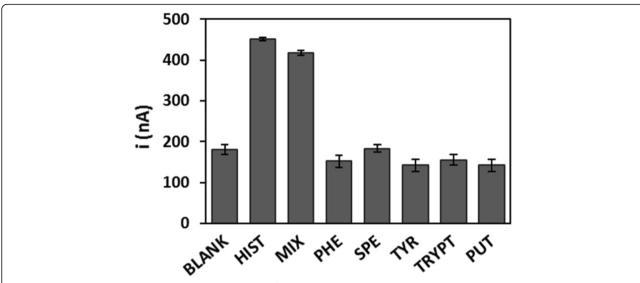


Fig. 4 Current intensities obtained for blank and for 25-mg L^{-1} solutions of histamine (HIST), phenethylamine (PHE), spermine (SPE), tyramine (TYR), tryptamine (TRYPT), and putrescine (PUT) and for a mixture containing 25 mg L^{-1} of each of these biogenic amines (MIX). Average values \pm SD obtained with three different sensors are represented

reduction of 7.5%), which indicates that the other biogenic amines do not significantly interfere in the analysis of histamine.

This demonstrates that the use of the redox mediator provides much better results in terms of selectivity towards histamine than when the analysis is performed through the reduction of the enzymatic products, as in our previous work (Torre et al. 2019), which showed signals for phenethylamine and spermine that were about 50% of the response for histamine.

Recovery studies

The feasibility of the sensor for the determination of histamine in real samples was assessed by recovery tests using spiked tuna and mackerel extracts (Table 2). The quantification of histamine was performed by recording chronoamperograms before and after spiking the extracts with a known amount of histamine. Recovery values of 96% and 97% were obtained for tuna and mackerel, respectively. These results demonstrate the accuracy of the sensor and its ability to perform histamine analysis in fish samples. Moreover, they also demonstrate the benefits of this design (DOX + redox mediator) for histamine analysis in fish extracts since this sensor is not affected by matrix effects. So, and compared to the sensor without the use of a

mediator (Torre et al. 2019), it is not necessary to construct calibration plots in fish extracts.

Conclusions

The importance of ensuring food safety has seen a huge growth due to consumer concerns about the food they eat. Therefore, the development of simple devices that allow rapid analysis to guarantee the safety of food is a field with increasing interest. In this work, a very simple sensor for histamine analysis was developed. The construction of this sensor only takes 30 min, and it only requires 40 µL of sample to perform the analysis. The sensor is able to determine histamine in a concentration range between 5 and 75 mg L⁻¹ with a good precision (coefficient of variation of 1.8%). Moreover, the sensor can be constructed and stored since it maintains nearly 88% of its initial signal after 35 days when stored at 4 °C. Its usefulness was demonstrated for histamine analysis in real fish extracts. The developed sensor fulfills several of the requirements of the current trends in analytical chemistry such as simplicity, low cost, small size, and requirement of low amounts of reagents and samples. Furthermore, the use of miniaturized electrodes (SPCEs) for the construction of the sensor and the

Table 2 Results of the recovery tests for the quantification of histamine in fish extracts. Average values \pm SD obtained with three different sensors are indicated

	Histamine before spiking (mg L^{-1})	Histamine added (mg L^{-1})	Found value (mg L^{-1})	Recovery (%)
Tuna	14.0 ± 0.2	40	52.4 ± 3.5	96 ± 8
Mackerel	13.4 ± 0.2	25	37.7 ± 1.4	97 ± 5

commercial availability of portable instrumentation (potentiostat) offer the possibility of decentralized analysis, which could be very useful for the control of histamine on-site along the whole fish production chain, from capture to consumption.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10. 1186/s40543-020-0203-3.

Additional file 1: Figure S1. Cyclic voltammogram of [Fe(CN)₆]⁴⁻. **Figure S2.** Chronoamperograms of different concentrations of histamine. **Table S1.** Figures of merit of the developed biosensor.

Abbreviations

CE: Counter electrode; CNTs: Carbon nanotubes; CV: Cyclic voltammetry/ voltammogram; DOX: Diamine oxidase; GA: Glutaraldehyde; GCE: Glass carbon electrode; HIST: Histamine; HRP: Horseradish peroxidase; ISE: Ionselective electrode; LOD: Limit of detection; LOQ: Limit of quantification; MIP: Molecularly imprinted polymer, n.r.: Not reported; NPs: Nanoparticles; PB: Phosphate buffer; PHE: Phenethylamine; PhotoHEMA: Photocured poly(2-hydroxyethyl) methacrylate; PUT: Putrescine; RE: (Pseudo)reference electrode; SPCE: Screen-printed electrode; SPE: Spermine; TRYPT: Tryptamine; TYR: Tyramine; WE: Working electrode

Acknowledgements

Not applicable

Authors' contributions

This work was conceived by RT and ECR and is included in the project "FishBioSensing" of which CDM is the principal researcher. ECR designed and organized the studies and RT performed the experimental work. The analysis of the results was carried out by RT, ECR, and HPAN. RT and ECR wrote the draft of the manuscript and HPAN and CDM revised it critically. CDM and HPAN were responsible for the funding acquisition and the project administration. All authors read and approved the final manuscript.

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Availability of data and materials

Research data have been provided in the manuscript and in the additional file.

Competing interests

The authors declare that they have no competing interest.

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