The Application of Polyurethane-LiClO₄ to Modify Screen-Printed Electrodes Analyzing Histamine in Mackerel Using a Voltammetric Approach

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ABSTRACT: Histamine is an important substance that can be applied as a parameter for allergic reactions and food freshness. This study develops a method to produce a histamine sensor based on electrodes modified using polyurethane-LiClO₄. A sensor method was developed where this sensor was produced from polyurethane. The application of 4,4′-diphenylmethane disiocyanate (hard compound) and palm kernel oil-based monoester polyol (soft compound) to produce polyurethane (PU) based on bio-polyol. The addition of lithium perchlorate (LiClO₄) was done in order to increase the conductivity of PU. The oxidation process was detected using cyclic voltammetry, whereas the electrochemical impedance spectroscopy was used to analyze the conductivity of the polymer. The polyurethane-LiClO₄ was attached on a screen-printed electrode (SPE) within 45 min. Moreover, the 1% LiClO₄-PU-SPE presented satisfactory selectivity for the detection of histamine in the pH 7.5 solution. The LiClO₄-PU-SPE presented a good correlation coefficient \( R = 0.9991 \) in the range \( 0.015 \text{–} 1 \text{ mmol·L}^{-1} \). The detection limit was 0.17 mmol·L⁻¹. Moreover, the histamine concentration of mackerel samples was detected by the PU-SEP-LiClO₄. Several amine compounds were chosen to study the selectivity of histamine detection using SPE-PU-LiClO₄. The interference was from several major interfering compounds such as aniline, cadaverine, hexamine, putrescine, and xanthine. The technique showed a satisfactory selective analysis compared to the other amines. A satisfactory recovery performance toward varying concentrations of histamine was obtained at 94 and 103% for histamine at 0.01 and 0.1 mmol·L⁻¹, respectively. The application of PU-SEP-LiClO₄ as an electrochemical sensor has a great prospect to analyze histamine content in fish mackerel as a consequence of PU-SEP-LiClO₄ having good selectivity and simplicity.

1. INTRODUCTION

Nowadays, food security becomes the main attention of the food industry due to various foods that have been produced caused by the high demand. Several factors influence the stability of food content such as the presence of bacteria, the storage and packaging methods, and the production of chemical compounds such as biogenic amines, particularly histamine. Histamine analysis in foods draws huge attention from several researchers because of not only the capability to poison the consumers but also the stability of histamine to withstand inside foods during the cooking or heating process.

Various methods have been developed by researchers in order to analyze histamine in various samples such as fish, meat, cheese, fruits, milk, etc. Liquid chromatography and gas chromatography are the popular techniques chosen by researchers based on their ability to determine histamine concentration. Furthermore, the ability of these techniques is unquestionable such as being very selective, sensitive, robust, and well established. However, these methods have several disadvantages such as being time-consuming, requiring various chemical reagents, high price, and the fact that merely people who study analytical chemistry can comprehend these machines. Furthermore, histamine is a less chromophore and unvaporized compound, thus the modification of histamine structure can be done by a derivatization process only.

These issues can be solved by electrochemical sensor application. They are very fast, inexpensive, have fewer chemical materials, and are easily used. The approaches of these techniques are different compared to chromatography techniques. In order to detect histamine, the sensors require a specific receptor, biological (enzymes) receptors, or chemical receptors. Nevertheless, the biological receptor of the sensor known as biosensor offers instability and expensive test
 kits. Several studies reported the application of enzymes is unstable owing to many factors that can affect it such as temperature and pH. Several foods also contain metals that can work as an inhibitor to inhibit enzyme activity. A chemical sensor is a better choice in order to avoid the use of biological receptors. Nowadays, screen-printed electrodes (SPEs) modified with conducting polymer have been developed for various electrochemical sensing. The SPE becomes the best solution owing to its frugal manufacture, tiny size, ability to be produced on a large scale, and can be applied for on-site detection. Polymers become an alternative to modify the screen-printed electrodes due to their electrical conductivity, ability to capture analyte by chemical/physical adsorption, large surface area, and making polymers that are very appealing materials from electrochemical perspectives. A natural polymer such as polyurethane has recently attracted analytical chemistry researchers owing to the fact that they have a prospect to be employed in electrochemical devices. Natural polymers have several properties that can be considered for electrochemical application such as easy handling during production, having no harm to the environment, low toxicity, cost effectiveness, and biodegradability. Platinum, gold, and carbon are common materials used by researchers in order to modify electrodes. Furthermore, carbon, gold, and platinum are expensive and should be modified before being applied as electrochemical sensors. Lithium perchlorate \( (\text{LiClO}_4) \) is an alternative in order to modify the conductivity of the polymer. Not only the price is cheaper but also the production of PU-LiClO\(_4\) is easy. In this study, \( \text{LiClO}_4 \) was employed owing to having lattice energy. It contains cations and anions. Furthermore, the ionic conductivity is affected by the size where the current is produced by the diffusion rate of anions and cations. Thus, it can be concluded that not only \( \text{LiClO}_4 \) is a good conductivity but also the production is easy. Furthermore, this material is a better solution compared to other materials (gold, carbon, platinum, and graphene) due to the interfacial resistance being low. Thus, \( \text{LiClO}_4 \) can be diluted in most solvents due to possession of dissociation energy.

Such advantages of SPE-PuLiClO\(_4\) encourage us to construct a new electrode for electrochemical sensing, and no study was published on histamine detection using SPE-PuLiClO\(_4\) by an electrochemical approach. Thus, this study can be considered as a new approach to electro-analysis of histamine. Histamine was then determined using a voltammetric approach. The sensor was applied to monitor the histamine concentration in fish mackerel.

## 2. EXPERIMENTAL SECTION

### 2.1. Materials and Preparation of SPE-PuLiClO\(_4\)

Palm kernel oil (PKO) was supplied by UKM Technology Sdn. Bhd. through MPOB/UKM Station Plant, Pekan Bangi Lama, Selangor and prepared using Badri et al.'s method. 4,4-Diphenylmethane diisocyanate (MDI) was acquired from Cosmopolyurethane (M) Sdn. Bhd., Klang, Malaysia. Lithium perchlorate \( (\text{LiClO}_4) \), polyethylene glycol (PEG) 400, and acetone solution were obtained from Sigma Aldrich Sdn. Bhd, Shah Alam and have been employed to produce SPE-PuLiClO\(_4\). Film production was produced using a casting method.

In this work, 10.0 g of palm kernel oil (PKO) and 2.0 g of PEG 400 were dissolved in 10 mL of the solution of 30% acetone and then stirred at ambient temperature for 5 min to acquire a homogenized solution. Afterward, 10.0 g of MDI was dissolved in 10 mL of 30% acetone and stirred. The MDI solution was poured slowly into PKO and PEG 400 solution in order to control the exothermic reaction, and stirred until the homogenized solution was obtained. Various amounts of 1, 3, and 5% of lithium perchlorate \( (\text{LiClO}_4) \) were employed and put into the mixture until a homogenized solution was acquired. The solution was poured onto the screen-printed electrode using the casting method and left at room temperature to dry the film. Thereby, SPE-PuLiClO\(_4\) was obtained.

### 2.2. Instruments and Chemicals

Metrohm Autolab Electrochemical Workstation (UKM, Bangi, Malaysia) (Figure 1) was used to conduct cyclic voltammetry (CV), differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) analysis. All electrochemical experiments were performed using three electrodes as SPE-PuLiClO\(_4\) as a working electrode, Ag/AgCl applied as a reference electrode, and platinum wire was employed as an auxiliary electrode. All
experiments were conducted at ambient temperature. Histamine (HIS), cadaverine (CAD), and putrescine (PUT) were obtained (≥99% purity) from Sigma Aldrich Sdn. Bhd. The supporting electrolyte was potassium chloride (KCl 0.1 M) (Sigma Aldrich Sdn. Bhd). Sodium hydroxide (NaOH) (0.1 N) and hydrochloric acid (HCl) (0.1 N) were used to control the pH solution and obtained from Sigma Aldrich Sdn. Bhd. The application of 0.1 M disodium hydrogen phosphate (Na2HPO4) and 0.1 M sodium dihydrogen phosphate (NaH2PO4) was used to produce the phosphate buffer solution (PBS) (0.1 mol L−1), and both materials were purchased from Sigma Aldrich Sdn. Bhd. All chemicals with analytical grade were employed during experiments. Solutions were prepared using deionized water cleansed with a Milli-Q system and stored at 4 °C.

2.3. Analytical Procedure and Real Sample Preparation. Histamine stock solution (10 mmol L−1) was established by weighing histamine (111 mg) and dissolving in 100 mL of PBS (0.1 mol L−1) at pH 7.5. A serial dilution was obtained from histamine stock solution ranging from 0.015 to 1 mmol L−1. Each concentration of histamine standard was analyzed using the CV method in potential ranging from 0 to +1.0 V.

Fish mackerel samples were bought from a fish store in Dengkil, Malaysia, and stored in a fridge. Briefly, 5 g of fish sample was scaled and homogenized using PBS (0.1 mol L−1) at pH 7.5 with 20 mL. The mixture was stirred for 5 min at 100 rpm and filtered using filter paper. Approximately 100 μL was transferred to a 10 mL volumetric flask and diluted to the tag with PBS (0.1 mol L−1) to produce the specific volume. Afterward, two histamine levels of standard solutions were chosen (0.13 and 0.015 mmol L−1) in order to study the proposed technique for histamine determination in fish samples.

3. RESULTS AND DISCUSSION

3.1. Deposition of PU-LiClO4 at the SPE Surface. The CV and EIS techniques were applied to the electrochemical behavior of histamine. It was studied at unmodified and modified electrodes with various amounts of LiClO4. The purpose of LiClO4 addition is to increase the conductivity of PU. Thus, due to the PU porosity, the lithium was entrapped inside the PU pores. The examination applied several pH values ranging from 6 to 8 in PBS (0.1 mol L−1). The investigations presented that the phosphate buffer was a satisfactory solution owing to the histamine appearance. Some studies mentioned that the application of PBS (0.1 mol L−1) as a supporting electrolyte to detect histamine in samples is the best solution due to the fact that it offers a better analytical response, whereas the application of sodium hydroxide (NaOH) and hydrochloric acid (HCl) was not suggested by several studies due to the fact that it can reduce the selectivity of histamine detection.28,29 The various concentrations of PBS (pH 7.5) as the supporting electrolyte at 0.01, 0.05, 0.10, and 0.50 mol L−1 were investigated. Nevertheless, the PBS levels did not disturb the histamine signal when detected using the CV method. Furthermore, the PBS (0.1 mol L−1) at pH 7.5 was chosen in several studies and discovered to be suitable due to a good signal during histamine analysis providing better sensitivity,28 thus it was chosen for further electro-analysis.

The conductivity of PU-LiClO4 was measured by the alternating current (ac) EIS method as shown in Figure 2 using a Metrohm Autolab Electrochemical Workstation (UKM, Bangi, Malaysia) with a frequency ranging from 1 Hz to 10 MHz at 100 mV amplitude under room conditions (25 °C, 1 atm). The unmodified electrodes such as SPE and SPE-PU and modified SPE such as SPE-PU-LiClO4 with various concentrations of lithium perchlorate were analyzed using impedance spectroscopy in PBS (0.1 mol L−1) at pH 7.5. The circuit model was fitted using Zview Analyzer Software.

The electrical conductivity (σ) was calculated from the bulk resistance (Rb), determined from the equivalent circuit analysis by using the Zview analyzer software, and the polymer film thickness (l) was measured using thickness gauge calipers and contact area of the thin film (0.25 cm2), in accordance with the equation shown below.30 By using eq 1, the conductivity of PU and SPE were 5.8 × 10−3 S·cm−1 and 1.2 × 10−3 S·cm−1, respectively. Meanwhile, the conductivity of SPE-PU-LiClO4 was 1.6 × 10−4 S·cm−1 due to the incorporation of lithium perchlorate.

\[
(\sigma) = \frac{l}{A \times R_b}
\]

Figure 3 presents the cyclic voltammograms of the unmodified electrodes such as SPE and SPE-PU and modified SPE such as SPE-PU-LiClO4 with various concentrations of lithium perchlorates amount in 0.1 mol L−1 KCl.

Figure 2. Spectra of the impedance of unmodified and modified electrodes in PBS (0.1 mol L−1) at pH 7.5 after being analyzed using Zview Analyzer Software.

Figure 3. Cyclic voltammograms of an unmodified electrode and modified electrode with various lithium perchlorates amount in 0.1 mol L−1 KCl.
applied in this research being a natural polymer produced from palm kernel oil and exhibiting a weak electrochemical response owing to the conductivity being low. Furthermore, the addition of LiClO₄ in this study was imperative in order to modify the conductivity of PU.

Figure 3 reveals an oxidation reaction of bare SPE and SPE-PU-LiClO₄ in KCl (0.1 mmol L⁻¹) with various concentrations of LiClO₄ (1, 3, and 5%) that can be indicated as an anodic peak and appeared at +0.5 V. The analytical response of bare SPE and SPE-PU-LiClO₄ ranged at −0.2 to +1.0 V, showing a specific potential at +0.5 V of both, whether the SPE and SPE-PU-LiClO₄. The unmodified SPE gave a larger current due to the SPE surface produced from the material that can release a high electrical current. Furthermore, compared to the modified SPE, the signal gave a higher current owing to the addition of LiClO₄. However, the application of LiClO₄ in this study did not release an oxidation process owing to the material not dissolving in polyurethane but being entrapped inside the PU pores.

The cyclic voltammograms obtained can be used to evaluate the electron transfer during analysis using the Randles–Sevcik formula. The cyclic voltammograms obtained can be used to evaluate the electron transfer during analysis using the Randles–Sevcik formula:

\[ I_p = 2.65 \times 10^{5} CD^{1/2}A v^{1/2}n^{3/2} \]  (2)

where \( C \) is the histamine level applied, \( n \) is the electrons transferred, and \( D \) is the diffusivity of 0.1 mmol L⁻¹ KCl. Furthermore, the SPE has a surface area of 0.2 cm² with 0.25 cm² for PU-SPE-LiClO₄ (0.5 × 0.5 cm). This area is called the geometric area of the working electrode. Thus, it can be seen that PU-SPE-LiClO₄ has more surface area than the SPE. Furthermore, the concentration of surface area or symbolized as \( (\tau) \) can be estimated using the equation below:

\[ I_p = A F n^{1/2} \left( \frac{R}{T} \right) \]  (3)

where \( I_p \) is for the peak current, while \( A \) is for the region of the electrode, \( v \) is the scan rate, \( F \) is Faraday’s constant, \( R \) is the ideal gas constant, and \( T \) is the experimental absolute temperature. Thus, according to the obtained results, polyurethane was combined with 1% of LiClO₄ in order to modify the screen-printed electrode. This modification was used for further studies such as a scan rate study, pH study, and analytical performance of the modified electrode.

3.2. Effect of Scan Rate. The scan rate was also studied using the cyclic voltammetry technique. This study wanted to investigate the anodic signal and potential of histamine at PU-SPE-LiClO₄ if various scan rates were applied. Figure 4 presents the cyclic voltammograms of histamines (0.063 mmol L⁻¹) in PBS (0.1 mol L⁻¹) at pH 7.5 on PU-SPE-LiClO₄, employing several scan rates (0.01–0.10 V s⁻¹). The peak current obtained shows a direct proportionality on the scan rate and potential applied, obtaining the equation \( i_p(\mu A) = 0.0551 + 214.58v \) (in mV s⁻¹) and \( R^2 = 0.9977 \), respectively. Thus, Figure 5a reveals that the histamine can be oxidized on the surface of the modified electrode.

The electrode involved in the process can be studied using the relationship between the scan rate, potential response, and anodic peak. Thus, the scan rate effects were studied in order to observe the histamine response during electrochemical analysis. The scan rates ranged from 0.01 to 0.10 V s⁻¹ using the CV technique. The obtained results show that the anodic peak of histamine increases when the scan rate \( v \) increases. The correlation can be described by the formula below:

\[ I_p(\mu A) = 0.051v^{1/2}(V^{1/2}s^{-1/2}) + 214.58(r = 0.9977) \]  (4)

Based on this research, the scan rate affected the oxidation peak of potential, where the histamine potential \( E \) was moved to a positive course. A satisfactory equation (Figure 5b) was obtained between the scan rate inverse or symbolized as \( (1/v) \) and the peak potential

\[ E_p(V) = 51.012 + 199.28 \ln v(Vs^{-1})(r = 0.9977). \]  (5)

3.3. Influence of pH. Based on this study, the heights and shapes of the histamine signal were affected by the pH value. The choice of pH level is a compulsory and imperative parameter that should be noted due to the fact that it can affect histamine redox. Several pH values were employed in order to observe the effect of pH at 0.25 mmol L⁻¹ of histamine using the CV technique, and pH ranged at 6.0–8.0 using 0.1 mol L⁻¹ PBS. Cyclic voltammetry was employed and ranged at 0.00 to +1.50 V, with 0.05 V s⁻¹ as a scan rate (Figure 6).

The acquired results reveal the effect of pH values in phosphate buffer solution. The connection between pH and peak potential of the supporting electrolyte is revealed in 6 and represented by using eq 5:

\[ E_p(V) = -0.0992 + 0.7206 \times pH \]  (5)

Here, 0.7206 V/pH (slope) indicates that there is an electrode reaction between protons and electrons. The signal of histamine increased with pH ranging at 6.0–7.5 and then decreased at higher values (Figure 6).

The analytical response of histamine analysis was satisfactory when the application of pH values at 6.5–7.5 was applied. Nevertheless, the PBS (0.1 mol L⁻¹) at pH 7.5 was selected owing to the better anodic peak current acquired. Furthermore, with a substantial anodic peak with pH 7 and pH 7.5, it can be concluded that there is a reaction between the modified electrode and histamine imidazole ring. The imidazole ring of histamine contains pKa at 6.04, and thus the reaction between the electrode and histamine imidazole ring is gradually decreased because the pH is 8 or higher.

3.4. Electrochemical Behavior of Histamine on Unmodified SPE and PU-SPE-LiClO₄. The sensitivity and selectivity of PU-SPE-LiClO₄ and unmodified SPE are studied
and compared in Figure 7. Histamine (1.0 mmol·L⁻¹) was detected using the modified electrode, and the CV technique was used and ranged at 0.0 to +1.0 V (0.05 V·s⁻¹ scan rate). The analytical responses of histamine were obtained and investigated on PU-SPE-LiClO₄ and unmodified SPE. The voltammogram of the PU-SPE-LiClO₄ released different redox couples with the unmodified SPE. Figure 7 shows a satisfactory oxidative peak of histamine that emerged at +0.31 V for the SPE-PU-LiClO₄, and no cathodic peak was found, which can indicate that this was an irreversible reaction. Meanwhile, the unmodified SPE signal released an oxidative peak at +0.58 V and it can be deduced that there was no possibility for histamine to entrap or react on the SPE surface whether by physical or chemical adsorption. Various studies have reported that the application of different voltammetries and electrodes that have been modified chemically or biologically will affect the electrode’s ability to analyze histamine and give different signals. A study reported that the application of the amperometry technique using a carbon electrode to analyze histamine released an anodic peak at +1.1 V.³⁴ Meanwhile, the different results were provided by another study when the DPV was applied and the carbon paste electrode (CPE) was used as the electrode, determining an oxidative peak of histamine at +1.30 V, while for a CPE modified using a single-walled carbon nanotube (SWCNT), the histamine anodic peak was found at +1.20 V.²⁸ Furthermore, the application of CPE modified by lignin showed an identical result at +1.20 V.³³ Nevertheless, the application of SPE modified by PU-LiClO₄ revealed a distinct anodic peak compared to other studies. Keow et al. (2007) studied and published the SPE application to analyze histamine in prawns, where according to their study, the SPE itself can be used to analyze histamine, so the electrode was modified using a photocuring method to produce a membrane to entrap enzyme immobilization and coat on the SPE surface.

Figure 5. (a) Calibration curve of the histamine oxidative peak vs the scan rate (0.01–0.10 V·s⁻¹) and (b) calibration curve between the current (µA) and potential applied (V).

Figure 6. Influence of pH values of histamine (0.25 mmol·L⁻¹) in PBS (0.1 mol·L⁻¹).

Figure 7. Cyclic voltammogram comparison between (a) SPE-PU-LiClO₄ and (b) unmodified SPE to analyze histamine (1 mmol·L⁻¹) in PBS (0.1 mol·L⁻¹) at pH 7.5.
Table 1. Several Studies of Histamine Detection Using Electrochemical Sensors and Biosensors

| electrode                        | technique       | LoD (mmol·L⁻¹) | linear range (mmol·L⁻¹) | references        |
|----------------------------------|-----------------|----------------|-------------------------|-------------------|
| diamine oxidase/carbon paste      | amperometry     | 3.5            | 0.1−325.9               | Keow et al. 35    |
| amine oxidase/screen-printed carbon | FIA/amperometry | 3.0            | 10−300                  | Telsnig et al. 34 |
| amine oxidase/horseradish peroxidase/carbon | chronamperometry | 0.18          | 0.4−2.4                 | Alonso-Lomillo et al. 39 |
| MWNT/glassy carbon electrode     | DPV             | 0.076          | 0.1−100                 | Goto et al. 40    |
| pyrroloquinoline-Quinone-modified GCE | amperometry     | 341            | 360−1530                | Young et al. 41   |
| lignin-modified GCE              | SWV             | 0.28           | 5−200                   | Degefu et al. 43  |
| SPE-PU-LiClO₄                    | CV and DPV      | 0.035          | 0.015−1                 | this research     |

The acquisition results showed a direct proportionality regarding histamine levels ranging at 0.015−1 mmol·L⁻¹. Using this data, this study was validated, and the data is shown in Table 2. The calibration curve was established and is shown in Figure 8.

Table 2. Validation Study of Histamine Detection Using DPV in PBS (0.1 mol·L⁻¹) at pH 7.5 on the SPE-PU-LiClO₄

| validation applied | value                  |
|--------------------|------------------------|
| potential applied  | +0.31 V                |
| linear range       | 0.015−1                |
| coefficient correlation (R²) | 0.9993     |
| intraday (RSD, %)  | 3.05                   |
| interday (RSD, %)  | 9.02                   |
| detection limit    | 0.035                  |
| quantitation limit | 0.105                  |

Figure 8. DP voltammograms of various histamine levels in PBS (0.1 mol·L⁻¹) at pH 7.5 on SPE-PU-LiClO₄.

The limit of detection (LoD) and limit of quantitation (LoQ) were calculated using the equation of (3.3 ·Sᵣ/b) and (10 ·Sᵣ/b), respectively. Sᵣ represents the standard deviation of the in Figure 9, revealing the histamine in various levels in PBS (0.1 mol·L⁻¹) at pH 7.5 using the SPE-PU-LiClO₄. The line and points obtained were representatives for the value of the correlation coefficient and standard deviations. The relative standard deviation (RSD) was quite satisfactory, below 9% for six replicate analyses of each concentration.

3.7. Limits of Detection and Quantitation. The limit of detection (LoD) and limit of quantitation (LoQ) were calculated using the equation of (3.3 ·Sᵣ/b) and (10 ·Sᵣ/b), respectively. Sᵣ represents the standard deviation of the
intercept, while \( b \) represents the slope of the calibration curve for the LoD concentration ranging from 0.015 to 1 mmol·L\(^{-1}\). The calculated LoD and LoQ were 0.035 and 0.105 mmol·L\(^{-1}\) for histamine, respectively.

3.8. Precision and Stability. The study of precision and stability in this study indicated that SPE-PU-LiClO\(_4\) application is not suggested after the 7th day due to the fact that the signal will reduce for each day; nevertheless, the selectivity is steady. SPE-PU-LiClO\(_4\) was studied in terms of stability by calculating the oxidative peak of histamine standard at 0.13 mmol·L\(^{-1}\). The evaluation and determination were done from the 1st day until the 20th day for each week by using an identical electrode that can be kept in a droughty place to obtain a steady SPE-PU-LiClO\(_4\) (Figure 10).

The obtained results revealed that the histamine signal was decreased from 198.25 to 176.49 μA, presenting a 10.97% distinction in analytical response on the 7th day contrasted to the oxidative peak value of the 1st day; however, the potential applied did not shift. Furthermore, the 28th day showed a 47.4% of reduction in current response compared to the first day. Thus, it can be deduced that the peak was gradually decreased and it confirms that the polyurethane-LiClO\(_4\) modified electrode can be used for 1 week owing to the SPE-PU-LiClO\(_4\) sensitivity being reduced day by day and the

![Figure 9](https://doi.org/10.1021/acsomega.1c06295) Calibration curve of various histamine levels ranging from 0.015 to 1 mmol·L\(^{-1}\).

![Figure 10](https://doi.org/10.1021/acsomega.1c06295) DPV voltammograms of histamine in PBS (0.1 mol·L\(^{-1}\)) at pH 7.5 on SPE-PU-LiClO\(_4\) on different days starting from day 1 until day 28, and analysis was applied each week. (a) Analysis for day 1, (b) analysis for day 7, (c) analysis for day 14, (d) analysis for day 21, and (e) analysis for day 28.
application must be evaded so the bias calculation of histamine level in real samples can be avoided.

Histamine (0.01 mmol-L\(^{-1}\)) was analyzed and employed to investigate the reliability of SPE-PU-LiClO\(_4\) during electroanalysis using the proposed method yet using different electrodes the similar surface area. Meanwhile, the precision of this technique was investigated in terms of intra- and interday investigations. The study of intraday was performed at two concentrations of histamine (0.1 and 0.01 mmol-L\(^{-1}\)) by six repetition analyses. The analysis was acceptable after the RSD was acquired at 3.05%. Meanwhile, the study of interday was performed by using the identical histamine level (0.1 mmol-L\(^{-1}\)) and using the identical modified electrode, and detection was done each day. The RSDs were acquired at 7.54 and 9.02\% at levels 0.1 and 0.01 mmol-L\(^{-1}\) of histamine, respectively, indicating the better approach of histamine analysis by the proposed method. Thus, it denoted that the production and histamine detection using SPE-PU-LiClO\(_4\) are quite reproducible and reliable.

### 3.9. Disturbances

The selectivity and sensitivity of the proposed method and the presence of several amines such as putrescine and cadaverine represented biogenic amines and several amines according to their carbon number bonded directly to the nitrogen atom such as aniline, xanthine, and hexamine as primary, secondary, and tertiary amines, respectively. All of them were investigated using histamine analytical signals as well.

Various amines were analyzed using the DPV technique that contained histamine at 1 mmol-L\(^{-1}\), and the other amines were analyzed at 1 and 2 mmol-L\(^{-1}\). Based on the voltammogram, it can be deduced that all amines applied in this study did interfere with the histamine signal. The obtained result of the peak height of amines applied in this study can be denied. Furthermore, putrescine and cadaverine did not affect the histamine signal because they emerged at different potentials to histamine. Even aniline emerged at +0.43 V (Figure 11), which is a primary amine, and it concluded no disturbances in the histamine signal. Meanwhile, other biogenic amines such as cadaverine and putrescine that are generally found in fish accompanied by histamine, satisfactory anodic peaks of them were found at +0.18 and +0.19, respectively (Figure 11), practically not provoking the anodic peak of histamine. Furthermore, hexamine and xanthine did not affect the analytical response of the histamine signal when they were analyzed at 1 and 2 mmol-L\(^{-1}\), respectively.

### 3.10. Implementation of the Developed and Validated Technique

The proposed method was applied for histamine analysis in real samples such as fish and canned mackerel. The fish sample was detected in six replicates. The development of histamine standard calibration was applied to determine the histamine concentration in mackerel quantitatively. Histamine was found in fish and canned samples. The histamine levels were found to be 17 μmol-L\(^{-1}\) (1.89 ppm) and 5.3 μmol-L\(^{-1}\) (0.59 ppm), respectively. The recovery study (accuracy) was studied using two histamine levels (0.1 and 0.01 mmol-L\(^{-1}\)). The recoveries ranged from 94 to 103\%, showing great accuracy. Furthermore, according to the regulation of the FDA, the fish samples analyzed in this study have no capability to poison during human consumption. In order to verify the detection in real samples, the performance was compared to other studies in terms of linear range and detection limit.

### 4. CONCLUSIONS

The research studies the electrochemical method validated and developed for the determination of histamine using SPE-PU-LiClO\(_4\). The PU film applied in this research has pores that can entrap histamine inside it. Furthermore, histamine exhibits not only an anodic peak at specific potential but also an irreversible reaction during analysis using a polyurethane-LiClO\(_4\) modified electrode. A satisfactory LoD and LoQ were also obtained in concentrations ranging at 0.015–1 mmol-L\(^{-1}\). Furthermore, the modesty of SPE-PU-LiClO\(_4\) production and deposition onto the surface electrode causing good accuracy was obtained, having a potential to detect histamine in real samples. Furthermore, the addition of LiClO\(_4\) into polyurethane improved the electro-analytical response of SPEs. PU has also the ability to adsorb histamine into its pores, causing histamine to release an oxidative peak compared to the unmodified SPE. Most of the common interfering compounds such as cadaverine and putrescine did not disturb the histamine signal. The validated method was demonstrated by analyzing histamine in fish mackerel and its product obtaining good accuracy with satisfactory RSD. The obtained results show that the validated DPV method proposes a great prospect for histamine detection as quality control of foods and beverages.

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**Figure 11.** DP voltammograms of histamine (1 mmol-L\(^{-1}\)) and the presence of other amines: (a) 1 mmol-L\(^{-1}\) of aniline, cadaverine, and putrescine and (b) 2 mmol-L\(^{-1}\) of aniline, cadaverine, and putrescine, respectively.
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Notes
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