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An electrooxidative technique to fast fabricate copper phosphate electrodes capable of integrating high performance liquid chromatography for the label-free detection of fish freshness

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ABSTRACT

A simple and fast one-step electrooxidative method has been developed to monolithically produce a copper phosphate ($Cu_3(PO_4)_2$) compound on a disposable copper tape, which can be integrated with high performance liquid chromatography (HPLC) for the estimation of fish freshness. The $Cu_3(PO_4)_2$ compound of flake-like nanostructures was formed by applying a first anodic peak potential at the copper tape for 10 min in a 1 M sodium dihydrogen phosphate (NaH_2PO_4) (pH 5.0) solution. The $Cu_3(PO_4)_2$ electrodes can detect the oxidative reaction of histidine and histamine in 20 mM NaH_2PO_4 solutions with pH 5.0–8.5. When integrating the electrodes with a flow injection system, the linear range and the calculated detection limit of histamine were respectively 2.5–250 ppm and 0.15 ppm. The electrodes integrated to HPLC can specifically detect the histamine concentrations in fish samples in the pH 7.5 NaH_2PO_4 solution, achieving an accuracy rate of 95.3% and a recovery rate of 101.1%.

1. Introduction

Detection of histamine is an important issue in the fields of food safety (Onal, 2007; Ordonez, Troncoso, Garcia-Parrilla, & Callejon, 2016) and allergy control (Niraj & Pandey, 2012). Histamine poisoning, such as scombrotoxicosis due to food spoilage, induces the allergy-like symptoms of headache, nausea and vomiting (Hungerford, 2010). Histamine is produced from the decarboxylation of free histidine by bacterial decarboxylase as foods decompose. The United States Federal Drug Administration (FDA) and European Union (EU) suggest that safe levels of histamine in fish meat should not exceed 50 ppm and 100 ppm, respectively (FDA, 2001; EFSA Panel on Biological Hazards, 2011). Therefore, developing a detector for the fast, easy and cost-effective detection of histamine is important for food safety.

Several methods have been developed to detect histamine, including surface-enhanced Raman spectroscopy (Janči et al., 2017), fluorometry (Cortacero-Ramirez, Arraez-Roman, Segura-Carretero, & Fernandez-Gutierrez, 2007), mass spectroscopy (Nei, Nakamura, Ishihara, Kimura, & Satomi, 2017;) and electrochemistry (Casella, Gatta, & Desimoni, 2001; Carralero, Gonzalez-Cortes, Yanez-Sedeno, & Pingarron, 2005; Stojanović, Mehmeti, Kalcher, Guzsvány, & Stanković, 2016; Sarada, Rao, Tryk, & Fujishima, 2000). Electrochemical detectors have great promise for mass production, good compatibility with portable instruments and easy operation. Current electrochemical histamine detectors can be divided into two categories: enzymatic and electrocatalytic electrodes. However, the immobilized procedures and stability of enzyme pose barriers to successful commercialization of enzymatic electrodes (Henao-Escobar, del Torno-de Román, Domínguez-Renedo, Alonso-Lomillo, & Arcos-Martínez, 2016). Although electrocatalytic electrodes can directly detect the oxidative response of histamine, the interference of other electroactive species in the biological matrix reduces sensing specificity and limits practical applications (Carralero et al., 2005). Therefore, developing an electrocatalytic electrode with high compatibility to a separating device is promising for the selective and specific determination of histamine.

According to our previous studies, copper phosphate ($Cu_3(PO_4)_2$) electrodes presented longer stability than copper oxide electrodes (Lee, Peng, & Wu, 2013; Lee et al., 2015), and can be operated in pH 5–10 solutions (Lee, Chang, & Wu, 2016). Several methods have been developed to fabricate $Cu_3(PO_4)_2$ electrodes (Wu & Shi, 2005; Lee et al., 2015). However, these fabrication methods are labor and time intensive. To the best of our knowledge, $Cu_3(PO_4)_2$ electrodes have not been used for histamine detection. Furthermore, $Cu_3(PO_4)_2$ electrodes are inexpensive to produce, making them conducive for use in

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disposable detectors.

The aim of this study was to propose a simple and fast one-step electrooxidative method to monolithically produce $Cu_3(PO_4)_2$ structures on a disposable copper tape, which can be used for the detection of histamine. The effects of electrooxidative parameters on the formation of $Cu_3(PO_4)_2$ compound are characterized by scanning electron microscope (SEM) and X-ray photoelectron spectroscope (XPS). The corresponding electrochemical properties are explored by cyclic voltammetry (CV) and hydrodynamic voltammetry (HDV). The electrode is integrated using commercial high performance liquid chromatography (HPLC) equipment to specifically detect histamine in real fish samples for the estimation of fish freshness.

2. Experimental

2.1. Reagents

L-Histamine, L-histidine, phosphoric acid, sodium dihydrogen phosphate (NaH_2PO_4) and perchloric acid ($HClO_4$) were purchased from Sigma-Aldrich (New Taipei City, Taiwan). Acetone and HPLC grade methyl alcohol was from Choneye pure chemicals (Taipei, Taiwan). Concentration-different histamine samples were prepared in 20 mM sodium dihydrogen phosphate (NaH_2PO_4) (pH 5.0) solution. Distilled water was obtained from a Milli-Q water purification system (Sigma-Aldrich, New Taipei City, Taiwan). All chemicals were of analytical grade. All solutions were filtered through syringe filters (pore size 0.22 μm; Millex-GP, Merck, Darmstadt, Germany) before being injected into an HPLC flow system.

2.2. Preparation of $Cu_3(PO_4)_2$ electrodes

The pristine Cu foil electrode was prepared by the previous study with minor modification (Lee et al., 2015). A piece of copper foil (99.9% Cu, 3 M, Taiwan) was adhered to a glass substrate to form a Cu electrode, and then covered with polyimide adhesive tape (1218 Tape, 3 M, Taiwan) as an insulator to define a disk-shaped working area with a diameter of 1 mm cut by a CO_2 laser. The pristine Cu electrode was ultrasonically cleaned in an acetone solution for 5 min, and then electroreduced to remove the native copper oxide/copper hydroxide layer using a cycling potential from −0.2 V to −0.6 V for 27.5 cycles with a 50 mV/s scanning rate in the 0.5 M $HClO_4$ solution. Subsequently, the electrocleaned Cu electrodes were placed in pH-varied 1 M NaH_2PO_4 solutions for scanning in the range of −0.2 V to +0.2 V, with a scanning rate of 10 mV/s to realize the oxidative mechanism of Cu electrodes with $H_2PO_4^-$ and protons. The pH values of 1 M NaH_2PO_4 solution were adjusted using 3 M phosphoric acid and 3 M sodium hydroxide. Finally, an adequate potential was applied to the Cu electrodes to form copper phosphate complexes. Following the electrodeposition process, the $Cu_3(PO_4)_2$-based electrodes were rinsed with distilled water and kept in air prior to use. The surface morphology and the chemical composition of the copper electrodes fabricated by different electrooxidative parameters were respectively characterized by SEM (JEOL JSM-7401F, Tokyo, Japan) and XPS (PHI 5000 VersaProbe, ULVAC-PHI Inc., Japan).

2.3. Real sample preparation

Following EU No 2073/2005 instructions (European Commission, 2005), we slightly modified the preparation method for real fish samples to extract free amino acids and histamine. Fresh mackerel (Scomber scombrus) was purchased from a local supermarket and then homogenized using a mortar and pestle until smooth and even in texture. Fish meat weighing 10 g was crushed and transferred to a 50 mL tube, then mixed thoroughly with 40 mL of distilled water in a tube. After homogenization and ultrasonication for 5 min, the mixture was centrifuged at 3000 rpm for 3 min. The supernatant was filtered through Whatman Grade OE66 filter paper (Sigma-Aldrich, Taiwan). The extracted solution diluted to 20% with 20 mM NaH_2PO_4 solution and then kept at -20 °C until analysis for histamine determination.

2.4. Electrochemical experiments and flow system integration

The electrochemical properties of the electrodes were explored by cyclic and linear sweep voltammetry with a potentiostat (CHI 842b, CH Instruments, Austin, TX) in a three-electrode system respectively using an Ag/AgCl (ALS Co., Ltd, Japan) electrode and a platinum (Pt) foil (Sigma-Aldrich, Taiwan) as the reference and the counter electrode. HDV was carried out in a flow injection analysis (FIA) system equipped with a peristaltic pump (DG-100N, Dogger, Taiwan), a manual sample injector valve (model 7725, Rheodyne, Berkeley, CA) with a 100 μL sample loop and a commercial flow cell (SF-100, Zensor R&D, Taiwan) to assess the oxidative potential of histamine in a flow system.

In addition, the electrode set in the same flow cell was connected with a Prevail™ organic acid column (5 μm particle size, 250 mm in length with a 4.6 mm inner diameter, Alltech, State College, PA), an intelligent pump (LC-10AT, Shimadzu, Japan) and an injection valve (7725i, Rheodyne, Berkeley, CA) with a 20 μL sample loop to form an HPLC system for histamine detection from real fish samples.

2.5. Statistical analysis

The current response and the retention time of histidine and histamine, expressed as the mean ± standard deviation, were calculated from at least three repetitions for each sample.

3. Results and discussion

3.1. Electrooxidative parameters of copper electrodes

This study offers a simple one-step electrooxidative method to monolithically produce copper-phosphate complexes on copper electrodes. Fig. 1 (a) shows the linear sweep voltammograms measured at the electrocleaned Cu electrodes from −0.2 V to 0.2 V with a 10 mV/s scanning rate in the 1 M NaH_2PO_4 solutions of pH 3.0–7.0. The oxidative peak current (I_{pa}) and potential respectively increased and positively shifted with the decrease of pH value. Nevertheless, no obvious anodic peak was observed in the pH 7.0 NaH_2PO_4 solution. Furthermore, the anodic peaks obtained in the pH 3.0–6.0 NaH_2PO_4 solutions can be divided into two groups. The first anodic peak potential (E_{pa1}) obtained in the pH 3.0, 4.0, 5.0 and 6.0 solutions was respectively about 0.145, 0.073, 0.007 and −0.038 V, and the second anodic peak potential (E_{pa2}) was respectively 0.155, 0.095, 0.045 and 0.017 V. In particular, the E_{pa1} and E_{pa2} obtained in the pH 3.0–5.0 solutions respectively had average decrements of about 69 mV/pH and 55 mV/pH. The E_{pa} shift with the change of pH values is not close to the 59 mV/pH of one proton-to-one electron transfer, suggesting the electrooxidation of copper electrodes is related not only to protons but also $H_2PO_4^-$. According to Aksu's study (Aksu, 2009), the reaction at E_{pa1} is mainly attributed to the oxidation of Cu^0 to form Cu^{I}_2O or $CuHPO_4$ as in Eqs. (1) and (2). The Cu^{I}_2O could be re-oxidized to $Cu_3(PO_4)_2$, $Cu_3(PO_4)_2{\cdot}3H_2O$ or $CuHPO_4$ as in Eqs. (3)-(6). At the E_{pa2} with the more positive potential, the Cu^{I}_2O can be electrochemically oxidized to $Cu^{II}O$ (Eq. (7)). The $Cu^{II}O$ can chemically react with $H_2PO_4^-$ and H^+ to produce $Cu_3(PO_4)_2$ (Eq. (8)). Moreover, the $Cu^{II}O$ may dissolve to form Cu^{2+} in acid solutions (Eq. (9)), and the Cu^{2+} combines with $H_2PO_4^-$ to form $Cu_3(PO_4)_2$ (Eq. (10)) and $CuHPO_4$ (Eq. (11)). It is worth noting that the I_{pa2} obtained at E_{pa2} in the pH 3.0 solution was much larger than that in the pH 4.0–7.0 NaH_2PO_4 solutions. This is attributed to the faster decomposition rate of $Cu^{II}O$ in more acidic solutions to increase the oxidative rate of Cu^{I}_2O to $Cu^{II}O$, inducing the larger current.

$$Cu_2O + 2H^+ + 2e^- = Cu + H_2O,\ E(vs.\ NHE) = 0.462-0.059\text{pH} \quad (1)$$