Development of Biosensor for Hypoxanthine Detection using Redox Polymer

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Abstract

Hypoxanthine (Hx) is an intermediate from the purine catabolism catalyzed by microorganisms during meat degradation, converting adenosine triphosphate into hypoxanthine. Biosensors based on a xanthine oxidase (XOD) enzyme are commonly used to determine the Hx amount in meat and estimate the meat's freshness. In this work, XOD, which is capable of catalyzing the oxidation of Hx, was modified on a screen-printed electrode by co-entrapping with an Os-complex modified redox polymer, allowing the detection of the amount of hypoxanthine. The influences of nanomaterials used in electrode modification and pH on the current response were studied. We found that the electrode modified with single-walled carbon nanotubes(SWCNTs), Os-polymer, and XOD provided the highest current response upon Hx oxidation. Moreover, the modified electrode demonstrated a large current in the presence of basic pH due to a favorable interaction between XOD and Os-polymer. Under the optimum conditions, the as-prepared XOD electrode showed a calibration graph with a linear range of 5-50 uM Hx. Application of the proposed electrode to estimate the Hx level in the fish meat sample was demonstrated.

Keywords - Biosensor, Hypoxanthine, Redox Polymer, Meat quality

Introduction

Meat freshness is an indicator of product quality for the food industry. After animal death, the microorganisms produce enzymes to digest the nucleotides in the muscle. Adenosine triphosphate (ATP) starts the degradation into hypoxanthine

An electrochemical biosensor is a promising analytical device for hypoxanthine determination due to its high sensitivity. The redox enzyme XOD selectively catalyzes the oxidation of hypoxanthine and is immobilized on the electrode surface as a bio-recognition element. When a redox reaction occurs upon the hypoxanthine oxidation, electrons are generated, yielding the electrical signal proportional to the hypoxanthine concentration.

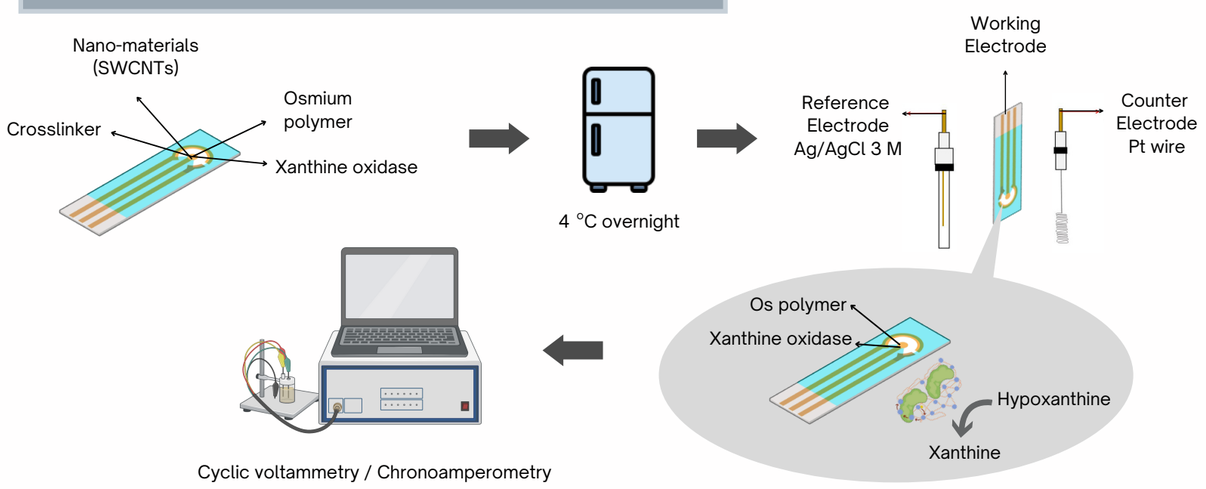
In this work, we investigate the performance of the biosensor based on xanthine oxidase for the detection of hypoxanthine. Single-walled carbon nanotubes and Os-complex modified polymer were used for biosensor preparation. The biosensor was studied for the suitable pH and applied to the analysis of hypoxanthine in fish meat.

Objectives

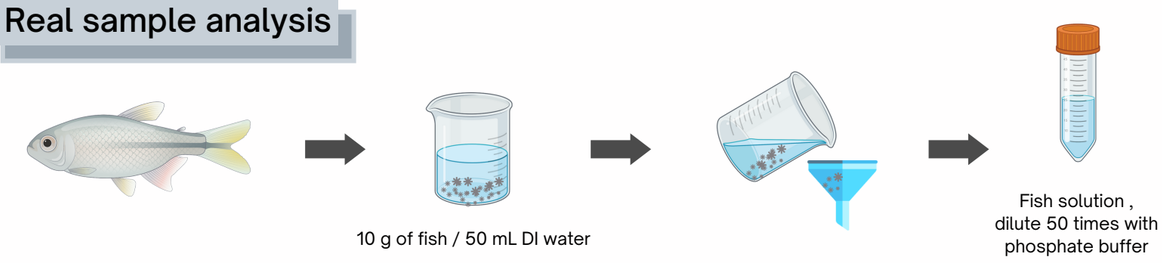
1. To develop a biosensor for hypoxanthine detection using the redox polymer.
2. To investigate the effect of nanomaterials (SWCNTs) for biosensor improvement.
3. To study the influence of pH on the catalytic current of the biosensor.

Method

Biosensor preparation and Instrumentations



Real sample analysis



Calibration graph : measure the current in fish solution
Standard addition : measure the current in fish solution by adding Hx 2, 4, and 6 µM

Result and Discussion

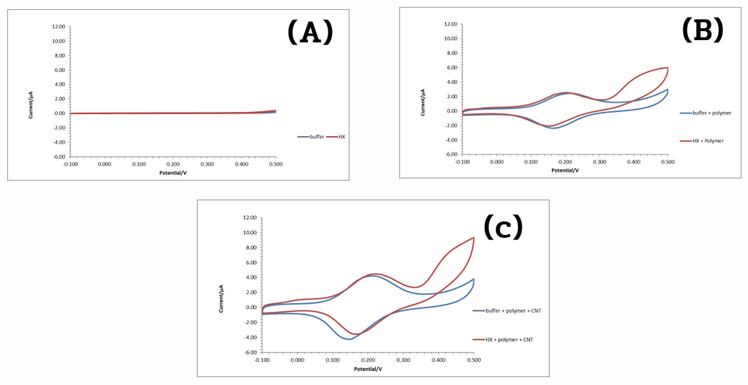


Fig.1 Cyclic voltammograms of the biosensor with different modifications: (A) XOD, (B) XOD/Os-polymer, and (C) XOD/Os-polymer/SWCNT in the absence (blue line) and the presence of 1 mM hypoxanthine in phosphate buffer pH 7.4 at a scan rate of 5 mv/s.(Reference electrode: Ag-pseudo reference electrode)

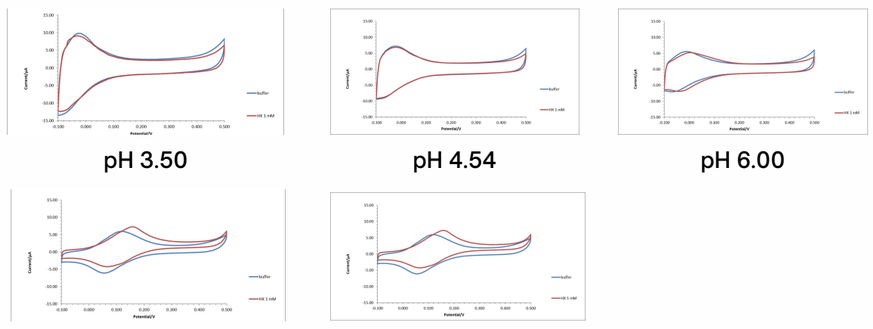


Fig.2 Cyclic voltammograms of the XOD/Os-polymer/SWCNT electrode in the absence (blue line) and the presence of 1 mM hypoxanthine (red line) at the varying pH with the scan rate of 5 mV/s.
(Reference electrode: Ag/AgCl 3 M KCl reference electrode)

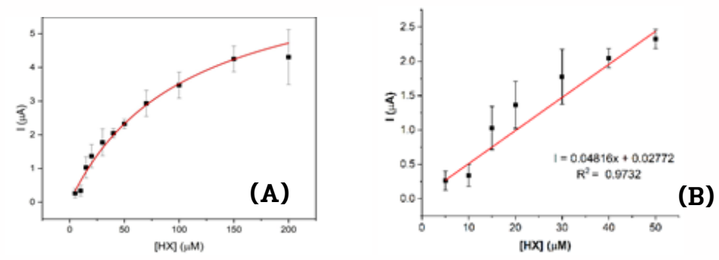


Fig. 3 (A) Dependence of the current response on hypoxanthine concentration for the XOD/Os-polymer/SWCNT electrode measured in phosphate buffer pH 8; applied potential = 0.28 V vs. Ag/AgCl 3 M KCl under stirring at 500 rpm. (B) Linear calibration graph for hypoxanthine determination (5-50 µM)

Table 1 Results of hypoxanthine content analysis in fish.

Method	[Hx]/ µM	mg Hx/1 g Fish
Calibration	6.109	0.208
Standard addition	6.958	0.237

Conclusion

The hypoxanthine biosensor was successfully fabricated based on the XOD enzyme. To improve the electron transfer of the biosensor, Os redox polymer and SWCNTs were utilized for electrode modification. Os-polymer offered improved current response while using SWCNTs provided a high current due to the large electrode surface area. The proposed biosensor could be used for hypoxanthine determination at basic pH, which promoted the favorable attraction between the enzyme and Os-polymer.

Reference

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[2] Mustafa, F.; Andreescu, S., Based Enzyme Biosensor for One-Step Detection of Hypoxanthine in Fresh and Degraded Fish. American Chemical Society 2020, 5 , 4092-4100