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Comparison of polypyrrole-based xanthine oxidase amperometric and potentiometric biosensors for hypoxanthine

A.T. Lawal*, S.B. Adeloju

NanoScience and Sensor Technology Research Group, School of Applied Sciences and Engineering, Monash University, Churchill, Vic 3842, Australia

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ABSTRACT

The preparation and performance of xanthine oxidase electrochemical biosensor developed for the determination of hypoxanthine is reported. Xanthine oxidase (XOD) and a mediator potassium ferrocyanide ($K_4Fe(CN)$) were entrapped into a polypyrrole (PPy) film during galvanostatic film formation. The optimum conditions for the formation of PPy-XOD-$Fe(CN)_6^{4-}$ film include 0.4 M pyrrole, 6.2 U/mL XOD, 40 mM $K_4Fe(CN)_6$, polymerisation period of 200 s, and an applied current density of 0.5 mA/cm^2. Two modes of detection, the potentiometric and amperometric modes, were investigated. The optimum potential for the amperometric biosensing of hypoxanthine was 800 mV vs. Ag/AgCl (3 M KCl) in 0.05 M phosphate buffer. A comparison of the sensitivities for the amperometric and potentiometric modes revealed that potentiometric detection was more sensitive and amperometric detection gave a wider linear concentration range. The PPy-XOD biosensor was successfully used for the determination of fish freshness.

1. 1 Introduction

Hypoxanthine (Hx) accumulates in biological tissue as it is the main metabolite of adenine nucleotide degradation in biological materials [1]. As soon as a fish dies or is caught, spoilage begins to occur. In fish muscle nucleotide, degradation commences at death and continues throughout storage. In the early stages, there is breakdown of adenosine-5-triphosphate (ATP) in fish muscle. This results in the release of adenosine-5-diphosphate (ADP) and further disintegration products, such as adenosine-5-monophosphate (AMP), ionosine-5-monophosphate (IMP), inosine (HxR), Hx, xanthine (X) and uric acid. Inosine monophosphate is one of the major contributing factors to the "pleasant" flavour of fresh fish whereas the accumulation of hypoxanthine/xanthine during storage contributes to the "off taste." [2]. The conversion of Hx to xanthine through the catalytic effect of xanthine oxidase (XOD) together with the production of H_2O_2 and reaction of O_2 was found to be the rate determining step in the overall reaction sequence in fish muscle. Thus, the concentrations of both inosine and/or hypoxanthine can be used as fish freshness indicators [1,3,4]. The amount of Hx present is often used as an index of freshness of meat in the food industries and the pathology of some processes in the human body [3,5]. Therefore, the determination of hypoxanthine (Hx) has considerable importance for quality control of fish and other fish products in the food industry [5–9].

Various methods for the quantification of hypoxanthine have been reported, such as spectrophotometry [10], chromatography and electrophoresis [11]. Some of these methods are tedious because they require pre-treatment and are complicated and time-consuming procedures. Electrochemical biosensors have been developed to enable a simpler and more rapid determination of Hx [1–3,12]. Many immobilisation methods of xanthine oxidase that have been used for this purpose include immobilisation of XOD in graphite [13], carbon paste [14], nylon mesh with glutaraldehyde (GLA) and covalent attachment to cellulose acetate [15]. XOD has been immobilised on membrane, nylon mesh [15]), on platinum and carbon paste electrodes [15,16]. XOD cross-linking with bovine serum albumin (BSA) and GLA [17] is the most commonly used method for the development of an amperometric Hx biosensor because of its ability to improve the stability of immobilisation [15,18,19] of any mediator. Dope sol–gel and laponite have also been used to immobilised xanthine oxidase for Hx determination (Yang [20]). Recently carbon nanotubes [8] have been co-immobilised with mediators such as methylviologen, hexacyanoferrate, hydroxylmethylferrocene [19] and colbaltphalcyamine [21] to enhance sensitivity and selectivity of the detection of Hx.

Direct electrochemical entrapment of XOD into polypyrrole is another approach, which has not been fully explored for the immobilisation of XOD. This method has been considered by few researchers for the immobilisation of XOD [22,23]. However,

* Corresponding author. Tel.: +61 242742664.
E-mail address: Abdulazeez.Lawal@det.nsw.edu.au (A.T. Lawal).

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electrochemical entrapment has been successfully used for the immobilisation of various enzymes, such as GOx [24,25], alcohol oxidase [26], urease [27], cholesterol oxidase [28], formate dehydrgenase [29], sulphite oxidase [30], and purin neucloside phosphorylase [31]

Enzymatic reactions that take place in the construction of these biosensors are commonly based on electrochemical oxidation of H_2O_2 produced in Eqs. (1) and (2) as follows:

$$Hx + O_2 \xrightarrow{XOD} Xanthine + H_2O_2 \quad (1)$$

$$Xanthine + O_2 \xrightarrow{XOD} + H_2O_2 \quad (2)$$

Different amperometric biosensors have been developed for the determination of Hx based on the above enzyme reaction [16,18,21,32–40] Amperometric-mediated biosensors have often been successfully used for the detection of hypoxanthine and xanthine [6,15,18,34–36,41], while limited number of potentiometric biosensors has been reported [42].

In this paper the preparation of Hx biosensor using electrochemical entrapment of XOD in polypyrrole film is presented. The performance of Hx biosensor will be explored for the fabrication of a stable, sensitive and selective Hx biosensor for rapid and reproducible measurement of fish freshness. Important considerations in the development of the Hx biosensor will focus on three significant aspects: (a) film formation and electrochemical entrapment conditions, such as current density, polymerisation time, effect of XOD and pyrrole concentration; (b) electrochemical modes of detections and (c) analytical utilisation of biosensor, interference and shelf life of the electrochemical biosensors.

2. Materials and methods

2.1. Reagents and standard solutions

Xanthine oxidase (XOD) (EC1.1.3.22 Grade 1) from buttermilk, potassium ferrocyanide, pyrrole and hypoxanthine were obtained from Sigma–Aldrich Chemical Pty Ltd. (NSW, Australia). Other chemicals used were reagent grade, and all reagents used in this work were prepared without further purification. XOD was stored in the refrigerator at 5 °C until required. The pyrrole was distilled under vacuum at 130 °C prior to use and this was stored in a closed bottle wrapped with aluminium foil in the freezer to prevent UV degradation until required for use.

2.2. Instrumentation

Electrochemical measurements were performed with a potentiostat/galvanostat designed and constructed in our laboratories. This instrument was used in the galvanostatic mode for the electropolymerisation. A three-electrode system, which consists of a platinum working electrode, a platinum wire counter electrode and a saturated calomel reference electrode (SCE), was employed for amperometric detection of hypoxanthine, while a two-electrode system, consisting of platinum working and a reference electrode, was used for potentiometric detection. The potentiostat was connected to a computer controller system. The solution was stirred when necessary with a Sybron Thermolyne (model S-17410) stirrer.

2.3. Preparation of XOD and KNO_3 electrodes

2.3.1. Electrode preparation

A 320 μm aluminium oxide powder was used to polish the platinum working electrode with a soft polishing pad, to remove any previous film and then finally polished with 5 μm aluminium oxide. The platinum electrode surface was washed thoroughly with Milli-Q water, rinsed under a stream of acetone and finally rinsed thoroughly with Milli-Q water to remove any of the remaining aluminium oxide. The electrode was dried with fibre-free tissue paper prior to use.

2.3.2. Electropolymerisation of PPy-XOD and PPy-NO_3 films

A three-electrode voltammetric cell was used to perform electropolymerisation of the PPy film. Platinum wire and Ag/AgCl (3 M KCl) were used as the auxiliary and reference electrodes respectively while the working electrode was a platinum electrode (0.17 cm^2). PPy-XOD was formed when xanthine oxidase (6.2 U/mL) and ferrocyanide was immobilised into the polypyrrole film by electropolymerisation of pyrrole in a solution which contained 0.1–0.5 M of the monomer and 20 mM potassium ferrocyanide at various current densities and a polymerisation time of 200 s. PPy-NO_3 was also formed when XOD and KNO3 were immobilised into polypyrrole film by electropolymerisation of pyrrole in solution which contain 0.4 M pyrrole and 0.05 M KNO3. After the galvanostatic film formation, the polymer electrodes were washed several times under a stream of Milli-Q water to remove any weakly bound XOD, KNO_3 or $K_4Fe(CN)_6$ molecules prior to use.

2.3.3. Amperometric and potentiometric measurements

Amperometric and potentiometric measurements were performed with a conventional three-electrode and two-electrode system, respectively. The amperometric measurement was performed in 50 mL measuring cells which contained 20 mL of 0.05 M phosphate buffer and stirred prior to measurement. A potential was applied and after current stabilisation (3 min), a standard analyte solution or sample was added and the current–time curve was recorded.

The potentiometric measurement was also performed in 50 mL cell, which contained 20 mL of 0.05 M phosphate buffer (pH 7.0). The enzyme and reference electrodes were immersed into the buffer solution. A zero current was applied and, after potential stabilisation (3 min), a standard analyte solution, or sample, was added to the gently stirred buffer and the potential–time response curves were recorded. The magnitude of the change in potential was correlated to concentration of analyte in the cell.

2.4. Determination of Hx

In the past, the quantitative Hx assay was performed by measuring the O_2 consumed or the H_2O_2 formed from the XOD catalysed reaction, as previously illustrated by Eqs. (1) and (2). The hydrogen peroxide is detected by amperometric measurement during oxidation of H_2O_2 at the enzyme electrode as follows:

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$

In this study, chronoamperometric measurement was performed in a 50 mL cell, which contained 20 mL 0.05 M phosphate buffer solution (pH 7.0). The enzyme electrode, the reference and counter electrodes were immersed into this buffer solution and a constant potential, 800 mV vs. SCE, was applied. After current stabilisation (about 2 min) a standard analyte solution, or sample, was added to the gently stirred buffer and the current–time response curves were recorded. The height, or the magnitude of the recorded current, was correlated to the concentration of analyte in the cell. In potentiometric mode, measurements were performed in 50 mL cell and buffer solution. The enzyme electrode and reference electrode were immersed into the cell. It is assumed that a potential couple is created by hydrogen peroxide at the enzyme electrode and the magnitude of the potential created was correlated to concentration of analyte in cell [43].

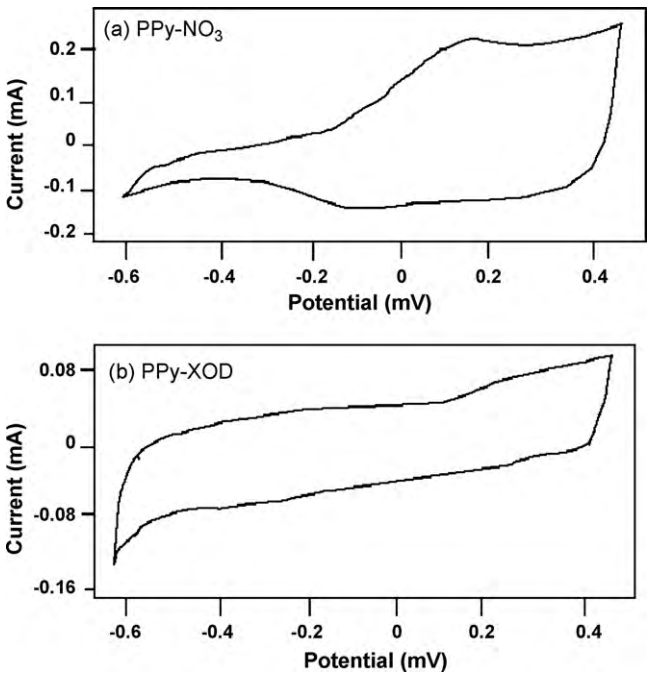

Fig. 1. CV of (a) PPy-NO_3 and (b) PPy-XOD films in 0.05 M phosphate buffer. The monomer solution contained 0.4 M pyrrole, 2.2 U/mL of XOD, current density: 0.5 mA/cm^2; polymerisation period: 200 s scan rate was 50 mV/s.

2.5. Hx determination in fish

Several extraction solutions, including perchloric acid [2], water [9] and trichloroacetictic acid (TCA) have been used to prepare fish samples for Hx analysis. Distilled water was used in this study. Many researchers realised that the tedious procedure of acid digestion was not necessary for measurement of hypoxanthine. Five grams of fish meat from Blue Grenadier fish fillet was homogenised in 20 mL water at room temperature and filtered through 0.2 μm filter membrane to obtain the fish extract in the filtrate. This was adapted for potentiometric biosensing of Hx. A mixture containing equal volumes of the fish extract and 0.1 M phosphate buffer was subjected to Hx analysis. The Blue Grenadier, Lake Entrance Flathead and Sword shark were analysed after storage under different conditions with the Hx biosensor.

3. Results and discussion

3.1. Cyclic voltammetry

Fig. 1(a) illustrates the cyclic voltammetry (CV) obtained for PPy-NO_3^- in 0.05 M phosphate buffer. The applied potential was scanned linearly from 400 mV to–600 mV at a scan rate of 50 mV/s. The characteristic oxidation and reduction couple of pyrrole appears at approximately at +0.1 and −0.1 V vs. Ag/AgCl, respectively. Fig. 1(b) shows the CV for PPy-XOD and the characteristic oxidation and reduction couple for pyrrole disappeared with substitution of nitrate ion with XOD. Incorporation of XOD into the PPy film may be responsible for the disappearance of oxidation and reduction couple and the decrease in PPy-XOD conductivity. The potential reached within the CV potential range was not positive enough to cause overoxidation; hence the reduction in conductivity can only be associated with the change in nature of the polymer after the incorporation of enzyme.

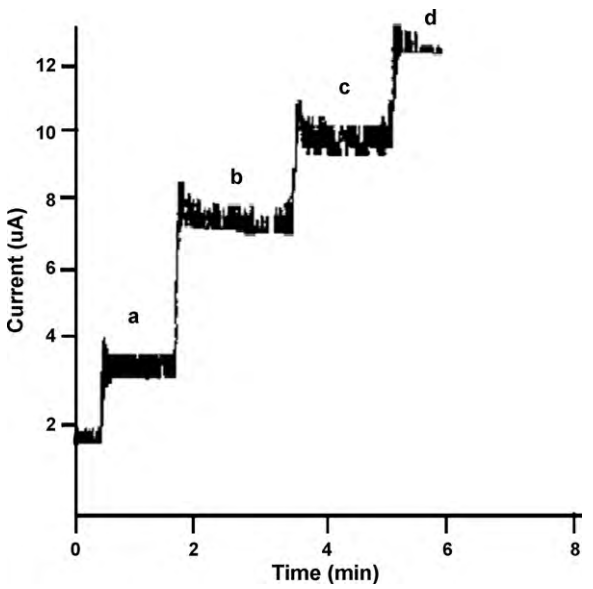

Fig. 2. Typical amperometric (current–time) response of PPy-XOD-$Fe(CN)_6^{4-}$ biosensor to Hx. (a) 10, (b) 20, (c) 40, and (d) 60 mM Hx. The monomer solution contained 0.4 M pyrrole, 2.2 U/mL of XOD, current density: 0.5 mA/cm^2; polymerisation period: 200 sec and applied potential was 0.80 V vs. Ag/AgCl electrode.

3.2. Response to Hx

The XOD-catalysed reactions taking place at the Hx biosensor have been illustrated in Eqs. (1)–(3). In the presence of molecular oxygen, XOD catalysed the oxidation of Hx and produced hydrogen peroxide, which can be detected by the PPy-XOD electrode in the presence of $Fe(CN)_6^{4-}$. The reduced mediator was simultaneously regenerated on the electrode surface giving an amperometric signal directly proportional to Hx concentration. Fig. 2 shows that the amperometric response increased with increasing Hx concentration. While in potentiometric mode potential is generated as a result of hydrogen peroxide produced from the electrode reaction.

The potentiometric response decreased with increasing Hx concentration. The change in potential (ΔE mV) of the PPy-XOD film upon exposure to Hx is due to H_2O_2 change in concentration. Similar result was obtained when H_2O_2 was produced in another biosensor [24]. However, the magnitude of the resulting responses was influenced by several factors, such as type and concentration of mediator, galvanostatic polymerisation conditions, pH and buffer concentration, XOD concentration and applied potential. Careful consideration of these factors was important for obtaining the optimum response for Hx. The specific observation from the study of each of these factors is discussed below.

3.3. Effect of galvanostatic polymerisation conditions

The chosen electropolymerisation time and current density can affect the thickness of polypyrrole film. It appears that the thicker the film produced the more $Fe(CN)_6^{4-}$ is incorporated into the conductive polypyrrole film and the higher the current or potential change produced. However, the response time to reach a steady state is longer for thicker films and thus the selection of an optimal thickness of polypyrrole film is necessary [2]. The effect of PPy layer thickness for film prepared in the presence of XOD on both amperometric and potentiometric response was thus examined. Evidently, the sensitivity of the response for Hx changed slightly with increasing polymerisation time. Polymerisation times less than 200 s resulted in an inadequate coverage of the platinum electrode; however beyond 200 s adequate coverage was achieved.

Table 1
Established optimum conditions for amperometric and potentiometric detection of Hx with PPy-XOD- $Fe(CN)_6^{4-}$ electrode.

Optimum condition	Amperometric mode	Potentiometric mode
[Pyrrole]	0. 4	0.4
$[K_4Fe(CN)_6]$	50 mM	50 mM
[XOD]	6.2 U/mL	6.2 U/mL
Film formation condition		
Polymerisation time (s)	200	200
Current density	0.75 mA/cm^2	0.75 mA/cm^2
Measurement condition		
Applied potential (mV)	800	0
Minimum detectable concentration [Hx] μM	6.0	4.5
Linear range1	0–50 μM	5–25 μM
Linear range 2	50–130 μM	

A polymerisation time of 200 s gave the best sensitivity. The results in Table 1 show that optimum applied current density between 0.5 and 0.75 mA cm^{-2}. Beyond this value, the sensitivity decreased and levelled out for film formed at applied current density greater than 1 mA cm^{-2}. Thicker films may contain a higher amount of the enzyme and mediator and may therefore exhibit higher sensitivity as long as the response is controlled by the enzymatic reaction. Decrease in sensitivity at a polymerisation time greater than 200 s (or 0.5 mA cm^2) could be due to the formation of a thicker film, which resulted in the formation of a higher diffusion barrier. A current density of 0.5 mA cm^{-2} was found to be optimum for the film formation.

Results in Table 1 show the optimum pyrrole concentration on the Hx response obtained with the PPy-XOD-$Fe(CN)_6^{4-}$ electrode. The sensitivity of the response increased rapidly with increasing pyrrole concentration reaching an optimum between 0.3 and 0.4 M pyrrole. The increasing sensitivity suggests that the amount of XOD entrapped increased with increasing pyrrole concentration. However, beyond 0.4 M of pyrrole the Hx response decreased slightly possibly due to the increased thickness of the polymer layer, which may result in an increased diffusion barrier. A pyrrole concentration of 0.4 M was subsequently used for the formation of the PPy-XOD-$Fe(CN)_6^{4-}$ layer prior to the measurement of Hx.

The sensitivity of the enzyme electrode increases as the enzyme loading is increased; however the sensitivity decreased when more than 6.2 U/mL of XOD was present. The platinum electrode was not completely covered with PPy-XOD film when electropolymerisation was performed in solution containing less than 6.2 U/mL; however beyond 6.2 U/mL there was an increase in film thickness. The increase could be as a result of the higher concentration of enzyme that balances the charge of PPy, thus enabling the formation of thicker film, which is less permeable to H_2O_2 and Hx. The chosen XOD concentration for all other work was 6.2 U/mL.

3.4. Comparison of amperometric and potentiometric detection

Various sensitive parameters that affect the sensitivity of PPy-XOD-$Fe(CN)_6^{4-}$ have been optimised as shown in Table 1. Some optimum conditions were chosen based on both reproducibility (measure of deviation from the mean value) and sensitivity (change in measured signal per unit concentration) of the responses. The use of amperometric and potentiometric modes was compared to identify the more sensitive and reliable sensing mode.

3.4.1. Amperometric detection

Fig. 2 shows the typical amperometric response for the PPy-XOD-$Fe(CN)_6^{4-}$ biosensor. It shows that the baseline for the response is not very smooth and that the increase in the response was not as distinct as for the potentiometric response. The calibration graph obtained for the amperometric response is linear between 0–50 μM and 50–130 μM, as shown in Fig. 3. These two regions enable measurements of both low and high concentrations to be made. The minimum detectable concentration of Hx with amperometric detection was 6 μM. The reason for the two regions may be due to saturation that was reached in the first region and new calibration commence immediately after that.

3.4.2. Potentiometric detection

Fig. 4 shows that the potentiometric Hx biosensor has a linear range from 5 to 20 μM. The minimum detectable concentration of

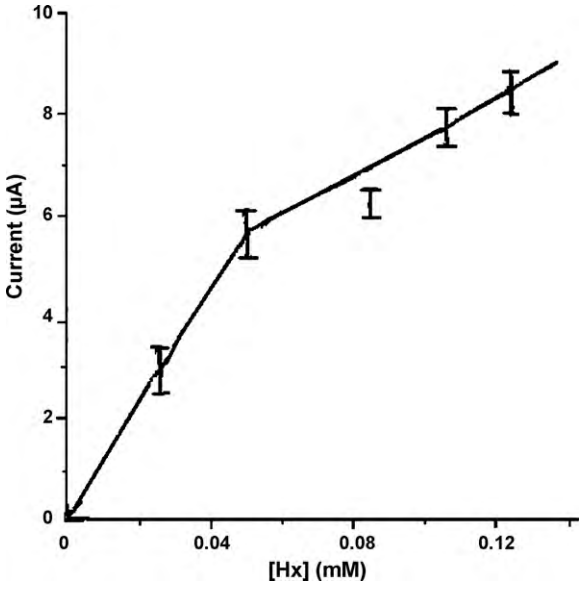

Fig. 3. Calibration plot obtained for the amperometric biosensing of Hx with PPy-XOD-$Fe(CN)_6^{4-}$ electrode. The monomer solution contained 0.4 M pyrrole, 6.2 U/mL of XOD, current density: 0.5 mA/cm^2 polymerisation period: 200 seconds, 800 mV and 40 mM $Fe(CN)_6^{4-}$.

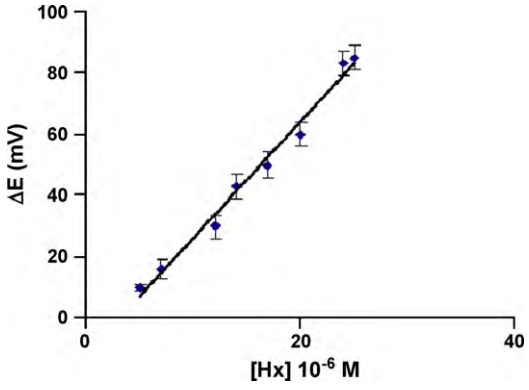

Fig. 4. Calibration plot obtained for the potentiometric biosensing of Hx with the PPy-XOD-$Fe(CN)_6^{4-}$ electrode.

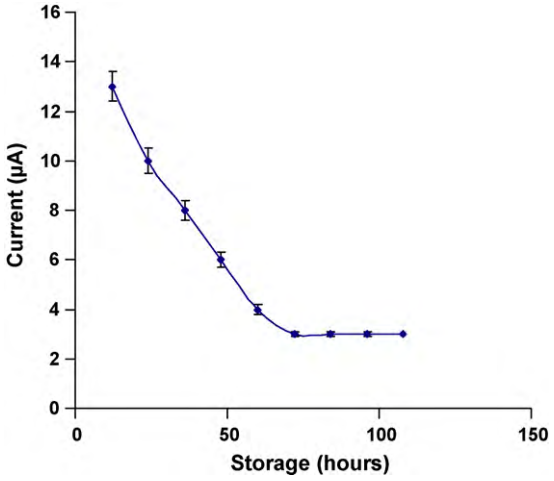

Fig. 5. Stability of the amperometric response of Hx obtained with the PPy-XOD-Fe(CN)$_6^{4-}$ electrode. [Hx] was 10 mM.

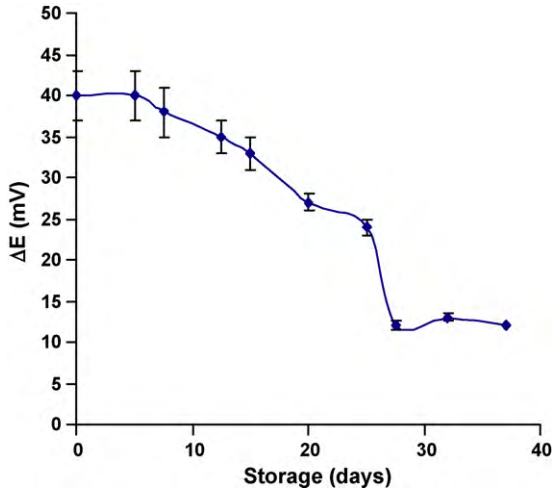

Fig. 6. Stability of the potentiometric response of Hx obtained with the PPy-XOD-Fe(CN)$_6^{4-}$ electrode. [Hx] was 10 mM.

Hx with of PPy-XOD-Fe(CN)$_6^{4-}$ electrode by potentiometric detection was 4.9 μM. Table 2 clearly shows that the potentiometric detection of Hx with PPy-XOD-Fe(CN)$_6^{4-}$ electrode enabled the detection of lower concentrations of Hx. Furthermore, the amperometric detection achieved a minimum detectable concentration of 6.2 μM. From the above results, sensitive quantification of Hx can therefore be achieved with potentiometric mode of detection.

3.5. Calibration curve and analytical application

A linear relationship was observed between the amperometric response and the Hx concentration between 0–50 μM and 50–130 μM, as shown in Fig. 3 This is similar to the observation made by Carsol et al. and Shan et al. [20,34] in previous studies. The two linear ranges are for sensitive determinations of Hx for both low and high concentrations of Hx. Saturation concentration point must have been reached in the first linear range. The minimum detectable concentration of hypoxanthine with the PPy-XOD-Fe(CN)$_6^{4-}$ biosensor in amperometric mode was 6.0 μM and the standard deviation at this level was 0.89 μM with $n = 4$. A linear relationship was also observed in Fig. 4 between the potentiometric response and the Hx concentration. The potentiometric response was linear from 5 to 20 μM with a correlation coefficient of 0.994 ($y = 3.79x - 11.61$ total 7 ponts of [Hx]). This is similar to that obtained by Shan et al. [20]. The minimum detectable concentration of Hx was 4.5 μM. This is approximately 16-fold lower than that of the oxygen-based biosensor developed by Watanabe et al. [3] and 100-fold lower than that developed by Balladin et al. [2] These linear ranges are suitable for the determination of Hx in biological and clinical samples.

Potentiometric biosensor was used in assessing fish and meat freshness. The quality of fish meat can be estimated from an increase in Hx concentration [3,9] and it has been reported that analysis based on Hx alone is an adequate indicator of freshness [2,44]. Table 2 shows the results obtained for the determination of Hx in three fish samples, namely Flathead, Blue Grenadier and Sword shark, with the PPy-XOD-Fe(CN)$_6^{4-}$ electrode based on potentiometric detection. Content of Hx was determined by standard addition method. The hypoxanthine found in the three different fish muscles ranged from 2.1 to 4.45 μmol/g. The results are within the range of values previously reported for common fish fillet by Zen et al., Balladin et al. and Nakatani et al. [2,9,45]. These results are evidence that the present sensor is a very effective analysis system and provides a simple and rapid method for the determination of Hx in fish samples. The agreement also suggests that the PPy-XOD-Fe(CN)$_6^{4-}$ electrode is useful for reliable, simple and economical detection of Hx as an indicator of fish freshness. As can be observed in Table 2, the amount of Hx increases with time of sample storage. This is similar to Hx content in fish obtained by Nakatani et al. [9] in a previous study.

3.6. Interference characteristics

Ascorbic acid (AA) and uric acid (UA) are considered to be major interferants in biological samples. The responses of the sensors towards AA and UA were greater in potentiometric mode than in amperometric mode when an applied potential of 800 mV vs. Ag/AgCl was employed. This could be reduced in amperometric mode using an applied potential of 0 mV or −200 mV vs. Ag/AgCl, and coating of nafion on the electrode to eliminate both AA and UA interferences in potentiometric and amperometric modes.

3.7. Stability of both biosensors response

The stability of the PPy-XOD-Fe(CN)$_6^{4-}$ electrode, as shown in Fig. 5, was very limited when used in the amperometric mode. The sensitivity of the response declined rapidly with increasing storage time up to 60 h and then stabilised thereafter with 77% reduction in sensitivity. The rapid loss in sensitivity may be due to the leaching of XOD and/or Fe(CN)$_6^{4-}$ from the electrode with increasing storage time. Alternatively, the decrease in sensitivity may be due to changes in the PPy film characteristic as a result of over oxidation at 800 mV. However, despite the reduction in sensitivity with time,

Table 2
Potentiometric analysis of Hx in fish with the PPy-XOD-Fe(CN)$_6^{4-}$ electrode.

Day	Lake entrance flat head (μmol/g)	Blue Grenader (μmol/g)	SwardShark (μmol/g)
1	2.10 ± 0.27	0.94 ± 0.05	1.53 ± 0.03
3	3.15 ± 0.65	1.81 ± 0.03	6.05 ± 1.6
5	12.5 ± 1.35	4.1 ± 0.90	6.82 ± 0.23
7	13.51 ± 3.50	4.45 ± 0.01	8.74 ± 0.69

the biosensor can still be used for routine determination of fish freshness, provided this is done by the standard additions method. The stability in potentiometric mode in Fig. 6 was much better and the sensitivity of the response did not start to decline until after 7 days. It lost over 50% of its initial response after 4 weeks. This could possibly be due to no current flows during potentiometric measurements, resulting in better stability than with amperometric measurements that use continued applied voltage which could be deleterious to its stability.

4. Conclusions

Amperometric and potentiometric biosensors for the determination of Hx have been fabricated by immobilisation of XOD and $Fe(CN)_6^{4-}$ into polypyrrole films by galvanostatic polymerisation. The optimum conditions for the formation of the PPy-XOD-$Fe(CN)_6^{4-}$ include 0.4 M pyrrole, 6.2 U/mL of XOD, 50 mM $K_4Fe(CN)_6$, a polymerisation time of 200 s and an applied current density of 0.75 mA/cm^2. The optimum applied potential for amperometric detection of Hx was 800 mV vs. Ag/AgCl (3 M KCl); however the potentiometric detection was more sensitive and enabled the detection of a wider linear concentration range of Hx. The biosensor had a minimum detectable concentration of 6.0 μM with amperiometric detection mode and 4.5 μM in the potentiometric mode. A linear concentration range for the biosensor in potentiometric mode was 5–25 μM, while the amperiometric mode had two linear ranges 0–50 and 50–130 μM. The biosensor was applied successfully to the determination of hypoxanthine in fish muscle. The concentration of hypoxanthine found in three different fish samples ranged from 2.1–8.7 μmol/g over a 7-day period. The results suggest that the biosensor can be reliably used for the assessment of fish meat freshness.

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