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FERROCENE MEDIATED AMPEROMETRIC BIOSENSOR FOR L-GLUTAMATE BASED ON L-GLUTAMATE OXIDASE IMMOBILIZED IN A PHOTOCURABLE METHACRYLIC FILM

(Biosensor Amperometrik L-Glutamat berperantara Ferosena berasaskan L-Glutamat Oksida Terpegun dalam Fotosalutan Filem Metakrilik)

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Abstract

L-glutamate is widely used as a flavour enhancer in various foodstuffs and seasoning in the form of monosodium glutamate (MSG). MSG has been linked to neurotoxic effects, metabolic disorders, headache, numbness and palpitation. In this work, an amperometric L-glutamate biosensor based on photocurable poly(2-hydroxylethyl methacrylate)-containing ferrocene film for the determination of L-glutamate in food samples is described. The sensor is fabricated based on simultaneous immobilization of both L-glutamate oxidase and ferrocene as a mediator during the deposition of poly(2-hydroxylethyl methacrylate) film via photocuring. Ferrocene was used to shuttle the electrons directly between the reduced enzyme and the electrode. From electrochemical studies, a linear response towards L-glutamate in the concentration range of 10-30 mM was obtained at applied potential of +0.25 V with the detection limit of 7.7 mM. The storage stability of the biosensor is up to 4 months under storage condition of 4 ^{o}C in refrigerator. The performance of the biosensor was applied to the determination of L-glutamate in food stocks from local supermarkets. Results from amperometric L-glutamate biosensor were further validated with HPLC method.

Keywords: photocurable, methacrylate polymers, ferrocene, L-glutamate biosensor

Abstrak

L-glutamat digunakan secara meluas dalam pelbagai bahan makanan dan perasa dalam bentuk mononatrium glutamat (MSG). MSG telah dikaitkan dengan kesan neurotoksik, gangguan metabolik, sakit kepala, rasa kebas dan berdebar-debar. Biosensor amperomerik L-glutamat berasaskan fotosalutan poli(2-hidroksiletil matakrilat)-mengandungi filem ferosena dihuraikan untuk

penentuan L-glutamat di dalam sampel makanan. Sensor ini direka berasaskan pemegunan secara serentak bagi L-glutamat oksida dan ferosena sebagai perantara semasa pemendapan filem poli(2-hidroksiletil metakrilat) menerusi fotosalutan. Ferosena digunakan sebagai perantara ulang-alik elektron secara langsung antara penurunan enzim dengan elektrod. Berdasarkan kajian elektrokimia, rangsangan linear terhadap L-glutamat diperoleh dalam julat kepekatan 10-30 mM pada keupayaan +0.25 V dengan had pengesanan 7.7 mM. Jangka hayat kestabilan biosensor penyimpanan dalam peti sejuk adalah mencapai sehingga 4 bulan pada suhu 4^{o}C. Prestasi biosensor diaplikasikan untuk penentuan L-glutamat dalam stok makanan daripada pasar raya tempatan. Keputusan daripada biosensor L-glutamat seterusnya disahkan dengan kaedah HPLC.

Kata kunci: fotosalutan, polimer metakrilat, ferosena, biosensor L-glutamat

Introduction

Glutamic acid is an important neurotransmitter of the central nervous system [1]. It is widely used as an additive to enhance the flavour in variety of foods prepared at home, restaurants, and by food processor in a form of monosodium glutamate (MSG). Glutamic acid exists both as free glutamate and bound with other amino acids in a protein. Only the free form of L-glutamate can enhance the food's flavour. The use of MSG in foods became controversial in the late 1960s when it was claimed to be the cause of a range of adverse reactions in people who had eaten foods containing the additive. Symptoms of MSG-sensitivity (called "Chinese restaurant syndrome") consist of numbness at the back of the neck and arms, weakness and palpitation. There is growing evidence linking the abnormalities of endogenous glutamate metabolism with certain diseases, such as Alzheimer's, Huntington's disease and Amyotrophic lateral sclerosis [2]. In Malaysia, the use of MSG must be in the form of 99.9% L-glutamate but there is no regulation on the limit of MSG that can be added into the food [3]. However, based on European Food Safety Authority (EFSA) panel after re-evaluating the safety of glutamate additives in 2017, the acceptable daily intake for glutamate additive has been set to 30 mg/kg bw/day [4].

There are several methods to determine the L-glutamate. Typically, separation techniques, in particular high-performance liquid chromatography [5, 6, 7], high-performance thin layer chromatography [8], thin layer chromatography and liquid chromatography-mass spectrometry [9] and capillary electrophoresis [10] are commonly used but require pre-concentration and sample derivatization. These techniques are also time consuming and need to be performed by highly trained operators. In contrast, simple and cost-effective spectrophotometric [11, 12, 13] and potentiometric [14] methods with chemical derivatization have been considered by some authors as an alternative for determination of L-glutamate in food. However, poor selectivity and interference from complex matrices in food may give inaccurate determination of L-glutamate. Therefore, enzymatic biosensors are gaining considerable attention due to their advantages of high selectivity and sensitivity towards L-glutamate**.** L-glutamate dehydrogenase [15, 16] and L-glutamate oxidase [17-23] had been widely used as a biological recognition to construct enzymatic sensors. Many enzymic methods have been used in conjunction with spectrophotometry [16, 19], potentiometry [20, 21] and amperometry [15, 17, 18, 22, 23] for the analysis of L-glutamate.

Enzyme electrodes have been proven to be the most widely applicable since it served to concentrate the enzyme activity in the vicinity of the sensor surface. A mixture of glutaraldehyde and bovine serum albumin (BSA) were usually used as a cross-linking reagent to immobilize the enzyme onto the electrode. A photo-cross linkable polymer polyvinyl alcohol bearing styrylpyridinium (PVA-SbQ) mixed with BSA and glutaraldehyde exposure has been reported for fabrication of an enzyme L-glutamate oxidase on a planar palladium electrode [18]. Hydrogen peroxide produced from the catalytic reaction by the L-glutamate oxidase which is proportional to the concentration of MSG was recorded at a potential of +400 mV versus Ag/AgCl reference electrode. The measurement range of L-glutamate was obtained between 50 nM - 100 µM. However, this method required a long procedure where glutaraldehyde in vaporized form was used instead of mixing directly to prevent enzyme deactivation during chemical attachment. Another approached was the

development of amperometric biosensor of L-glutamate based on the co-immobilization of L-glutamate dehydrogenase (GLDH) and salicylate hydroxylase (SHL) entrapped in a poly(carbamoyl) sulfonate (PCS) hydrogel and sandwiched between a dialysis membrane and a Teflon membrane on a Clark-type oxygen electrode [15]. Enzyme solution of GLDH and SHL was mixed with PCS and polymerization then achieved by adjusting the pH of the enzyme matrix through the addition of polyethylenimine (PEI). The mixture of the enzyme-PCS gel was spread over a Teflon membrane, followed by drying and storing at 4°C overnight. Simple immobilization technique through entrapment of the L-glutamate oxidase in chitosan film also required a few hours to complete the immobilization process [17].

In this work, the enzyme L-glutamate oxidase (Glox) was used as a biological recognition element to detect L-glutamate. The enzyme reactions with L-glutamate are illustrated in Equation 1. L-glutamate reacted with Glox producing H_2O_2, α-ketoglutarate, NH_3 and a reduced form of Glox. The product H_2O_2 is an electroactive species, which can be oxidized to yield current flow. However, due to the high oxidation potential of H_2O_2 which gives poor selectivity and interferences to the biosensor signal, therefore H_2O_2 was not used in the transduction process. Instead, ferrocene (Fc) was used as a mediator to shuttle electrons directly between the reduced enzyme and the electrode (Equation 2). The Fc diffused to the working electrode, oxidized or reduced at a fixed potential until it reached equilibrium (Equation 3). In this way, the current generated was directly proportional to the concentration of L-glutamate. The similar concept had been reported to construct glucose biosensor [24].

$$\mathrm{L-glutamate} + \mathrm{Glox_{oxidized}} \rightarrow \mathrm{Glox_{reduced}} + \alpha\mathrm{-ketoglutarate} + \mathrm{NH_3} + \mathrm{H_2O_2} \quad (1)$$

$$\mathrm{Glox_{reduced}} + 2\,\mathrm{Fc^+} \rightarrow \mathrm{Glox_{oxidized}} + 2\,\mathrm{Fc} \quad (2)$$

$$\text{At the working electrode: } \mathrm{Fc} \leftrightarrows \mathrm{Fc^+} + \mathrm{e^-} \quad (3)$$

A one-step procedure for fabrication of a L-glutamate biosensor film is using a photocuring technique. Briefly, both Fc mediator and Glox are immobilized in a polymer which is formed from photopolymerization of 2-hydroxylethyl methacrylate (HEMA). This is a simple and rapid technique for preparation of a biosensor, as compared with those described elsewhere [22, 23].

Materials and Methods

Materials and reagent

All chemicals used were of analytical grade and deionized water was used throughout for all solutions preparation. Ferrocene was obtained from Fluka (98% purity), 2-hydroxylethyl methacrylate (HEMA, 98%) purity) and L-glutamate oxidase (Glox) (≥ 5.0 unit/mg) were obtained from Sigma. Initiator 2,2-dimethoxy-2-phenylacetophenone (DMPP, 99% purity) was supplied by Aldrich and monosodium glutamate monohydrate was supplied by Acros Organic (99% purity). Phosphate buffer (0.1 M, pH 7.0) used as electrolyte in electrochemical measurements was prepared in 0.1 M NaCl.

Preparation of biosensors

Carbon-paste screen-printed electrodes (SPE) (Scrint Print Technology, Malaysia) were used as working electrodes with electrode surface 4 mm in diameter. Initially, a stock homogeneous solution of HEMA containing DMPP and Fc was prepared by mixing 1 mL of HEMA solution, 1.6 % DMPP (w/w) and 1 % Fc (w/w) in a dark bottle. The stock solution was stored at 4°C when not in used. The formulation was changed accordingly when certain experimental parameters were investigated. Then, appropriate amount of stock HEMA solution was mixed with Glox and a volume of 5 µL mixture solution of Fc/HEMA/Glox was deposited on the working electrode area and then exposed to UV radiation (λ = 365 nm, 15 W) under nitrogen gas in 8 minutes to form a solid thin layer membrane.

Electrochemical measurements

The electrochemical measurements were performed with an Autolab PGSTAT 12 Potentiostat/Galvanostat. A one compartment cell with a working volume of 4 mL 0.1 M phosphate/NaCl buffer was used. The working

electrode is a SPE coated with polymer-containing Fc film whereas silver/silver chloride (Ag/AgCl) and a glassy carbon electrode were used as reference electrode and auxiliary electrode respectively. The characterization of the potential-dependent behaviour of Fc with cyclic voltammetric (CV) was carried out by scanning between -0.5 - 0.7 V (versus Ag/AgCl) at a scan rate 5 mV/s in an unstirred 0.1 M phosphate/NaCl buffer pH 7. The effect of the detection potential on the electrode sensitivity was examined amperometrically for applied potentials varying between +0.1 - +0.4 V under a stirred solution of 4 mL 0.1 M phosphate/NaCl buffer pH 7.0, 30 mM L-glutamate.

The biosensor was optimized with regards to several parameters, namely, effect of pH, Fc and enzyme loading over the amperometric response of the electrode at an optimum applied potential of +0.25 V versus Ag/AgCl. For the determination of the effect of pH value on the biosensor response, 30 mM L-glutamate solution was prepared by adding 60 µL of 2 M L-glutamate with 4 ml of buffer with different pH (5 - 9). The stock pH 5 - 9 buffer solution was obtained by mixing 0.1 M phosphate solution with NaCl and pH was adjusted with NaOH or HCl. The effect of the Fc concentration used as a mediator on the biosensor response was tested by varying the concentration of Fc at 0.5%, 1% and 2% (w/w) and for enzyme loading, the amount of Glox was varied in the range of 20 - 160 U/g Fc/HEMA at the fixed amount of Fc and pH.

The stability of the biosensor for reusability (repetitive usage) was tested by preparing six different biosensors which three of them were used once a day and another three were used once in every two weeks. The performance for each biosensor was measured by using 20 mM standard L-glutamate. The biosensor performance of the first day was used as a reference and the percentage of the relative response was determined. After each usage the biosensors was stored in a closed container under dry storage at 4ºC. The reusability was carried out until the percentage relative response reached below 50% [25]. For the storage stability, another three biosensors were prepared and they were stored dry at 4ºC. The biosensors were tested periodically once a month for six months using 20 mM L-glutamate solution.

The calibration measurements were performed by placing the working electrode in 4 mL of a stirred 0.1 M phosphate/NaCl buffer (pH 7.0) at an applied potential of +0.25 V versus Ag/AgCl. When the background current reaching the steady value, an appropriate amount of L-glutamate solution was added into an electrochemical cell. After the addition, the current increased to a new steady value. The L-glutamate concentrations were added then plotted against the difference in current between before and after glutamate addition.

Biosensor at the optimum condition was applied to determine the concentration of the L-glutamate in food samples. The interference effect of the matrix in the samples was tested first by measuring any possible amperometric response after adding three possible interference species such as ascorbic acid, L-aspartate and D-glutamic acid into the samples. The interference study was also carried out by doing recovery study. For recovery studies, the biosensors were employed for the determination of L-glutamate in four different food samples such as fried rice seasoning, fish sauce, sour curry paste and concentrated chicken stock. Two set of food samples were prepared which one without spiked standard L-glutamate and the other one with spiked standard L-glutamate. Percent recovery was calculated by the formula below:

$$\% \text{ recovery} = (x-y)/z \times 100$$

x = average [L-glutamate] in spiked sample
y = average [L-glutamate] in non-spiked sample
z = average [L-glutamte] that was spiked in the sample

Food stocks were obtained from local supermarkets. Solid samples and pastes were dissolved in boiling water according to the manufacturer's instructions and treated as liquid samples. Samples were filtered using filter paper type Whatman Ashless before the determination. The liquid sample produced well-defined amperometric responses similar to those of L-glutamate standard.

HPLC method for L-glutamate determination

For HPLC method, the sample was centrifuged at 3000 rpm for 15 minutes and filtered using 0.4 µm membrane filter. L-glutamate levels were determined by isocratic liquid chromatographic using fluorescence detection after pre-column derivatization with o-phthaldialdehyde (OPA) [26]. OPA (108 mg) was dissolved in 1 mL of 99.5% ethanol. Borate buffer (0.4 M, pH 10.4) was added and followed by 20 µL of 2-mercaptoethanol and 20 µL of L-glutamate/sample made up to 10 mL. The derivatized L-glutamate solution was injected into the HPLC column within one minute after preparation. The HPLC analysis was carried out using a Jasco PV-1580 instrument with a ThermoHypersil-Keystone Hypersil ODS column (250 × 4.6 mm, 5 µm) at room temperature. The excitation and emission wavelengths of the Jasco FD-1520-Intelligent Fluorescence detector were set at 330 nm and 455 nm respectively to detect the derivatized L-glutamate. The volume of the derivatized sample injected was 20 µL. The analysis was carried out isocratically using a mobile phase of methanol-phosphate buffer (35:65, v/v) at pH 5.8.

Results and Discussion

The cyclic voltammetry study of immobilized ferrocene mediator in the biosensor

As shown in Figure 1, as the potential becomes more positive and the reduced form of the Fc is converted into the oxidized ferricenium species, hence there is appearance of the anodic peak in the range of +0.2 - +0.5 V. As the potential is reversed, a cathodic peak is detected in the range of +0.1 - +0.3 V attributed to the electrochemical activity of the ferricenium generated during the forward scan. The similar pattern was also observed in an earlier study using the similar polymer films with Fc as a mediator [24]. It can be shown that the immobilised Fc mediator demonstrated electrochemical reversible behaviour. Therefore, effect of applied potential on the biosensor response against L-glutamate was tested in the range of +0.1 – +0.5 V and the results are shown in Figure 2.

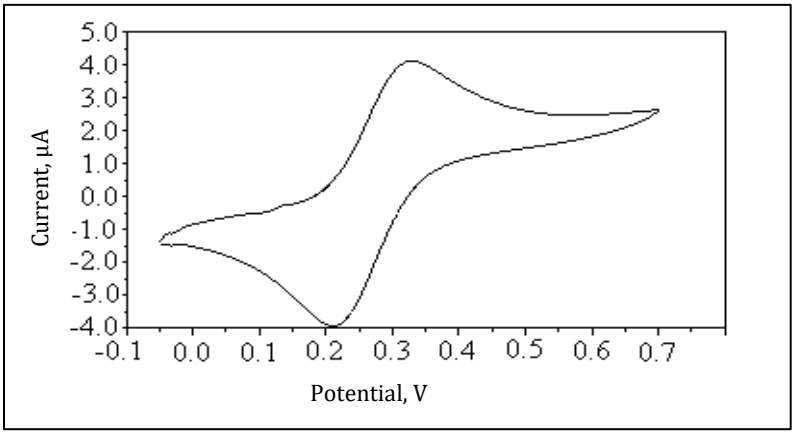

Figure 1. Cyclic voltammograms of Fc/HEMA/Glox biosensor versus Ag/AgCl electrode from -0.5 – +0.7 V with a scan rate of 5 mV/s in an unstirred 0.1 M phosphate buffer/NaCl pH 7

The biosensor response showed rapidly increasing with the applied potential until it reached at optimum potential of +0.25 V. At the potential higher than +0.25 V, the current was observed to decrease. The obtained potential was relatively low compared to reported potential by Liu et. al. [23] that was +0.6 V. The low applied potential was beneficial to avoid any interference from other electroactive species such as

ascorbic acid. Therefore, an applied potential of +0.25 V versus Ag/AgCl was carried out for all subsequent studies.

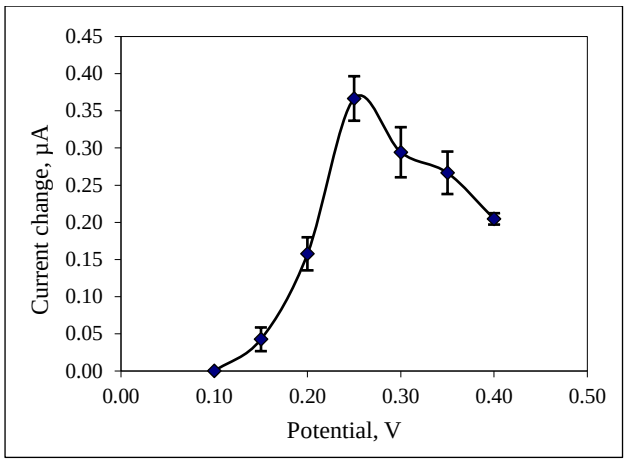

Figure 2. Effect of applied potential on the biosensor response studied for amperometric measurements for 25 mM L-glutamate in 0.1 M phosphate/NaCl buffer pH 7.0 versus Ag/AgCl electrode

Effect of pH

The influence of the buffer pH is very essential to the sensitivity of the biosensors because the pH does not only affect the electrochemical behavior of Fc but also the bioactivity of Glox. The bioactivity of the immobilized enzyme with Fc/HEMA/Glox that deposited on the electrode was influenced by the pH of the buffer used as shown in Figure 3. All buffers were solutions of 0.1 M phosphate buffer/NaCl. A plot of the current response to a fixed amount of L-glutamate as a function of pH change indicated that the response reached maximum value at pH 7.0. The behavior of the enzyme Glox was reported to be more stable in pH between 7.0 - 7.4 [27]. Therefore, further studies of the biosensor response with L-glutamate will be carried out at pH 7.0.

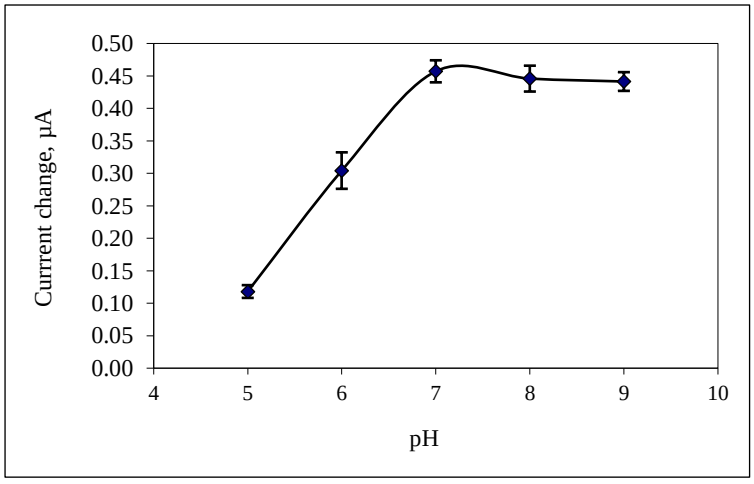

Figure 3. Effect of pH on the maximum response of the glutamate biosensor in 30 mM of L-glutamate at +0.25 V in 0.1 M phosphate/NaCl buffer pH 7.0 versus Ag/AgCl electrode

Effect of ferrocene concentration

Ferrocene as a mediator was used in this constructed biosensor. Ferrocene was immobilized together with Glox/HEMA deposited on the working electrode. In the absence of mediator, the biosensor did not demonstrate any response to the present of L-glutamate. This may be attributed to the non-existence of redox reaction of the H_2O_2 at the surface of the working electrode. In the presence of the Fc mediator, the one-electron oxidation of Fc to the stable ferricenium cation was a simple electron transfer reaction, where complications arising from adsorption or associated chemical reaction which were not expected [28]. Ferrocene has been used as the mediator for the determination of glucose using glucose oxidase as a biological sensing material [24]. Glucose oxidase and Glox belong to the same oxidase family with the same cofactor (FAD) with almost the same enzyme conformation [29].

The effect of varying concentration of immobilized mediator Fc on the biosensor response can be seen in Figure 4. The optimum concentration of Fc to give the best biosensor response to L-glutamate is at 1% of Fc by weighting of the biosensor film. The Fc concentration above 1% did not increase the biosensor response but instead resulting a decrease of response signal. This was probably due to the blockage of the access to the active site of the enzyme by excessive Fc molecules in the biosensor membrane. Ferrocene concentration of 0.5% was not enough to oxidize back the reduced enzyme and interact with L-glutamate substrate. These scenarios were agreeable with Wang et al. [30], when the mediator concentration was low, the biosensor response was limited by the enzyme-mediator kinetic whereas as the concentration was higher, the biosensor response was limited by the enzyme-substrate kinetics.

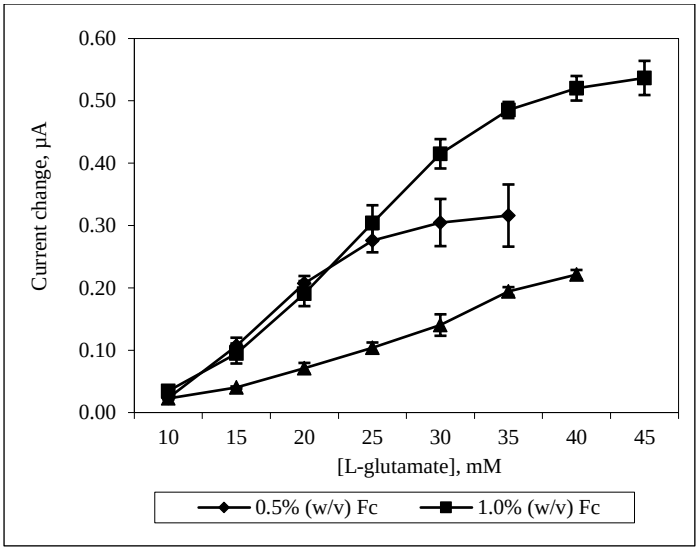

Figure 4. Effect of Fc concentration on the biosensor response at +0.25 V for a series of L-glutamate concentration in 0.1 M phosphate buffer/NaCl pH 7.0 versus Ag/AgCl electrode

Effect of enzyme loading

Enzyme loading also affected the performance of the biosensor. The Glox enzyme was used as a biological reagent for the detection of L-glutamate substrates in Fc/HEMA membranes. From the study of Glox enzyme loading (20 - 160 U/g Fc/HEMA), 120 U/g was enough to give the best performance for the biosensor (Figure 5). The insert graph was the plot enzyme loading versus current change in 40 mM L-glutamate. Reaction rates increased linearly with the increasing concentration of enzyme if the concentration of the substrate was in excess when compared with the amount of enzyme [31]. The sensitivity and the linear dynamic range of the biosensor increased with the increase amount of immobilized enzyme [32]. However, large immobilized enzyme loads were also considered unsuitable because

the limited space in the polymer caused the enzyme active site to be shielded and cannot be fully exposed to glutamate oxidation and reduction reactions [33]. Therefore, an enzyme load of 120 U/g HEMA was selected in this study for the preparation of all biosensors for further amperometry studies.

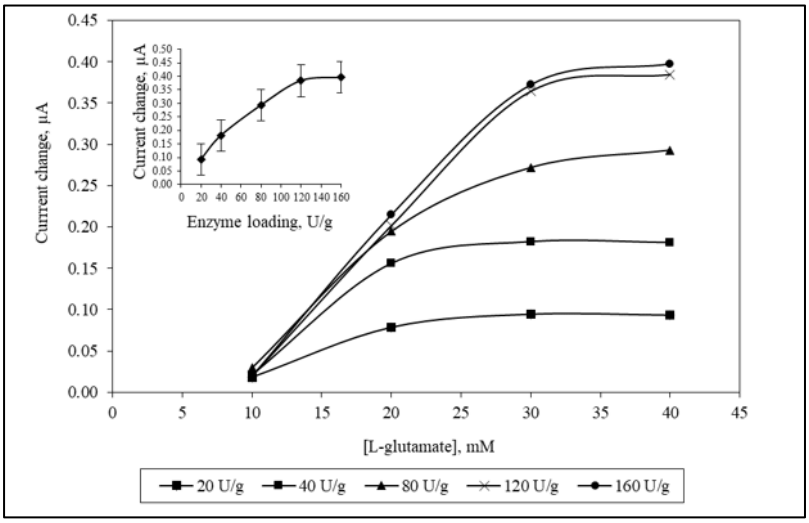

Figure 5. Effect of enzyme loading on the biosensor response at +0.25 V for a series of L-glutamate concentration in 0.1 M phosphate buffer/NaCl pH 7.0 versus Ag/AgCl electrode. The insert graph was the plot enzyme loading versus current change in 40 mM L-glutamate

The stability of L-glutamate biosensor

Stability characteristics related to reusability (repetitive usage) and shelf life are the important factors need to investigate. The stability of a biosensor was influenced by factors such as the method of enzyme immobilization and biosensor storage conditions. From the study of the stability by using the same biosensor for repeated measurement (Figure 6), the percent relative response showed an increasing trend from day 1 – 4 due to conditioning steps until its stabilized. This is associated with decreasing diffusional barriers of the analyte and membrane [25]. The percent relative response was very stable upon repeated injection from day 4 - 9, demonstrating that the membrane did not undergo fouling by the oxidation products. However, the percent relative response started to decrease from day 10 and reached below 50% at day 17. The decreasing trend of the biosensor may be due to hydrophilic property of the HEMA and its swell after it immersed in aqueous L-glutamate solution. The swelling process caused more L-glutamate substrate to penetrate the membrane. This condition would cause the enzyme activity to decrease due to the overexposure to the glutamate solution. Lim and Ang [31] also reported that the enzyme activity was decreased when exposing to high substrate concentrations. The swelling process of polymer membranes affected diffusion, surface properties, mechanical properties and surface movement [34].

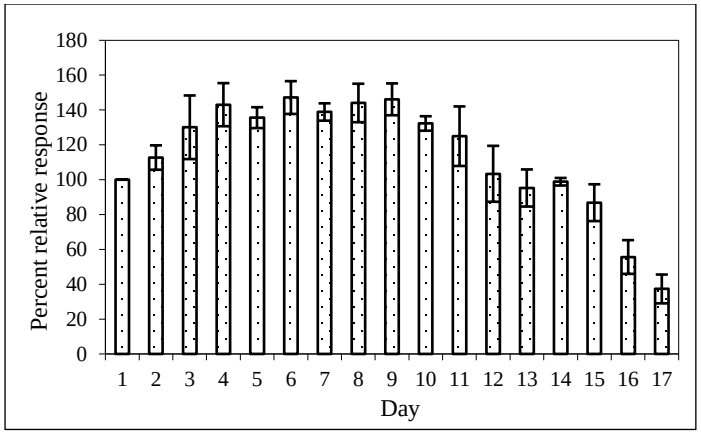

Figure 6. The stability of glutamate biosensor by using the same biosensor for reusability by taking the measurement once a day and kept dry at 4ºC while not in use

The stability of the biosensor was also tested by measuring the percent relative response in 2 weeks' time (Figure 7). The measurement on the first day was used as a reference and considered 100% performance. After measuring, the biosensor was stored dry at 4 ºC. The usage on the 75th day showed that the percent relative response decreased significantly by 41%. The finding showed that, if the biosensor did not use in everyday but only once in two weeks' time, the stability of the biosensor can be extended.

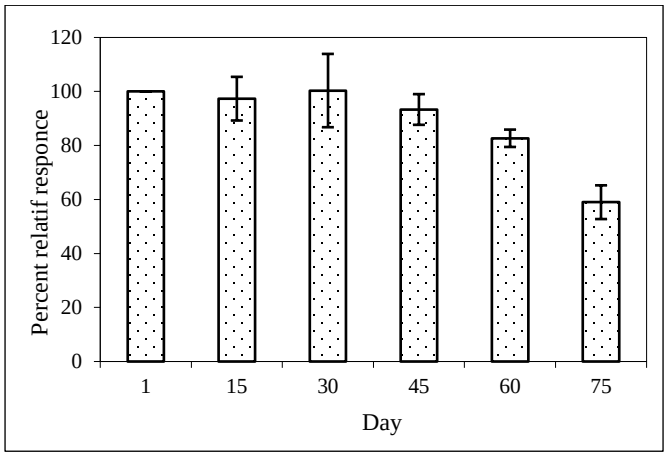

Figure 7. The stability of glutamate biosensor by using the same biosensor for reusability by taking the measurement once in two week's time and kept dry at 4ºC while not in use

Figure 8 showed the performance of a biosensor prepared in a similar preparation, stored at a temperature of 4ºC in a close container and dry state. According to Chang et al. [18], the storage stability of biosensors can be improved if the working electrode containing the immobilized enzyme is stored in a dry state (not dipped in a buffer solution). The first day measurement was used as a reference for the subsequence measurement. Biosensor using different Fc/HEMA/Glox membrane was measured only once at 1-month intervals and it showed a good performance up to 4 months of storage. A decrease in the performance of the biosensor after 4 months of storage, may be because of drying process of the membrane, even though it was not in used. Since the

biosensors were kept in drying state, continuous drying of the Fc/HEMA/Glox membrane in turn causing it to be unable to maintain enzyme activity. Water molecules was trapped in the Fc/HEMA/Glox membrane and were considered important in maintaining the enzyme activity.

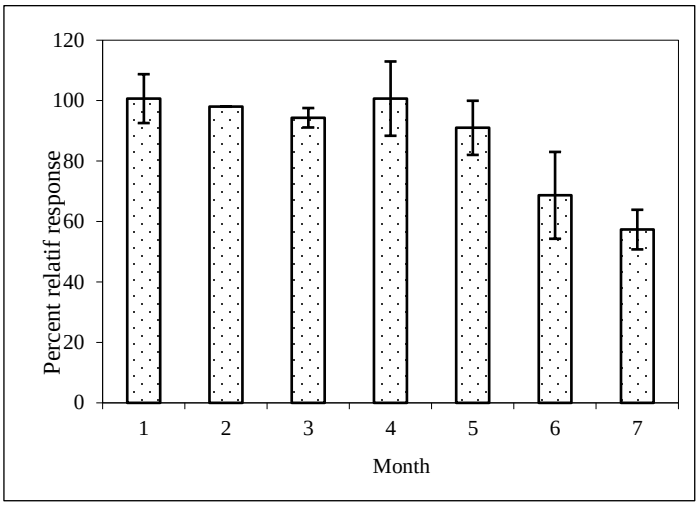

Figure 8. The storage stability of biosensor by taking the measurements once a month of different biosensor in 20 mM L-glutamate

The analytical performance of the L-glutamate biosensor

The electrochemical response of the biosensor to increasing concentration of L-glutamate is shown in Figure 9. A linear response to L-glutamate from 10 – 30 mM was demonstrated, with a sensitivity slope of 0.0173 μAmM^{-1} (R^2 = 0.984). The corresponding detection limit calculated from the L-glutamate concentration that producing an amperometric signal equals to the signal of the baseline plus three times of the standard deviation value [35] was 7.7 mM. The reproducibility of each data point had a relative standard deviation (RSD) value between 2.6% - 11.2% (n = 3) and this indicated that the biosensor showed reproducible responses.

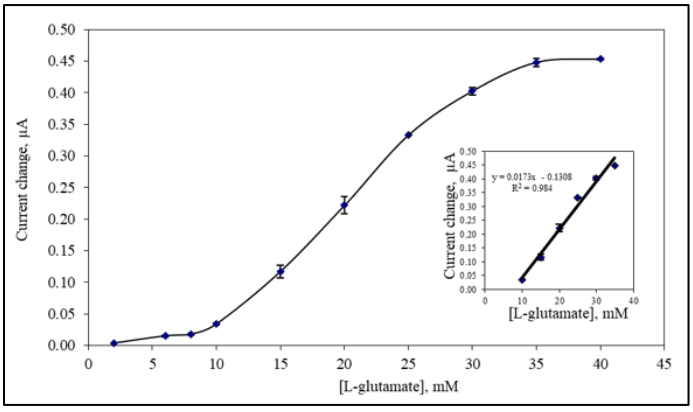

Figure 9. Response of the L-glutamate biosensor with changes in the concentrations of L-glutamate determined at applied potential +0.25 V versus Ag/AgCl electrode in stirred solution of 0.1 M phosphate/NaCl buffer pH 7. The linear range is also shown by the inset

The proposed glutamate biosensor was compared with the reported glutamate biosensor in literature. Performance of the proposed biosensor (Table 1) resulted the limit of detection which slightly higher than those of biosensors reported in literature. However, the proposed biosensor in terms of the fabrication is simple and faster. Moreover, the amount of L-glutamate in food sample was relatively high; in the range of 6.7 – 658 mg/100 g in fresh food to 0.05 – 6830 mg/100 g in processed food [36] and in food seasoning 4.8 – 21.3% [37]. Therefore, the proposed biosensor was practical to be used as a tool to detect the presence of L-glutamate in food.

Table 1. Characteristics of some glutamate biosensors reported in the literature

Type of Enzyme	Biosensor Configuration (Operational Mode)	Detection Mode	Linearity (mM)	LOD (mM)	Reference
Glox	Photocurable poly(2-hydroxylethyl methacrylate)/ferrocene	+0.25 V vs Ag/AgCl	10 – 30	7.7	Proposed
Glox	Absorption chitosan	+0.4 V vs Ag/AgCl	5×10^{-4} – 2×10^{-1}	1.0×10^{-4}	[17]
Glucose oxdase	Cross-linking glutaraldehyde/BSA/Nafion/ metal viologen	-0.65 V vs. SCE	Until 0.75	2×10^{-2}	[29]
Glox	Cross-linking glutraldehyde/BSA	+0.7 V vs Ag/AgCl	Until 0.8	3×10^{-3}	[38]
Glox	Nafion/Prussion blue	+0.0 V vs Ag/AgCl	1×10^{-4} – 1×10^{-1}	1×10^{-4}	[39]

The specificity of the biosensor to L-glutamate was evaluated by measuring a standard solution of possible interferents such as ascorbic acid, L-aspartate and D-glutamic acid under the same conditions. Ascorbic acid was chosen to represent any interference from carboxylic acid group species, L-aspartate for amino acid group and D-glutamate is a chiral to L-glutamate. No response was observed demonstrating that these substances did not interfere with the detection of L-glutamate.

Recoveries ranging from 98 –105% were obtained to the determination of L-glutamate using the L-glutamate biosensor in four different types of food matrices; this indicated that the complicated matrices of these food samples did not interfere with the performance of the glutamate biosensor.

The results of a comparative study for L-glutamate determination in some food samples using both the biosensor and standard HPLC procedure are shown in Table 2. When compared with the HPLC method, the content of L-glutamate determined in the selected food items which was analyzed by the biosensor and demonstrated a very close result. A statistical analysis (*t*-test) at 95% confidence level comparing the means of measurements obtained from the two methods of glutamate sensor and HPLC also showed no significant difference, indicating that the method developed was in good agreement with the established method.

Table 2. Comparison of L-glutamate level in food stocks obtained from local supermarkets by using the L-glutamate biosensor and HPLC method (n=3)

Type of Food	L-Glutamate Level (%)		
	Biosensor	HPLC	t-test
Anchovies stock cube	17.70 ± 0.27	17.73 ± 0.29	0.1
Fried rice mix-chicken seasoning	21.24 ± 0.46	21.38 ± 0.48	0.4
Vegetable soup seasoning	5.75 ± 0.09	5.66 ± 0.15	0.9
Fried vermicelli paste	5.47 ± 0.17	5.57 ± 0.06	1.0

Conclusion

The use of Fc mediator together with photocurable membrane technology in the fabrication of L-glutamate biosensor has successfully produced an amperometric L-glutamate biosensor (applied potential of +0.25 V versus Ag/AgCl, pH electrolyte 7.0, 1% ferrocene concentration, 120 U/g enzyme loading and with up to 4 months shelf life) that can be used to detect L-glutamate in food items. The photocurable technique in this work was simple and faster compared to covalent bonding or absorption technique reported in the literature. The biosensor exhibited a reproducible characteristic with acceptable RSD values. The biosensor had developed a linear dynamic range of 10 – 30 mM L-glutamate with LOD 7.7 mM. L-glutamate level in food samples determined from the biosensor was comparable to conventional HPLC method.

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