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A Novel Potentiometric Membrane Sensor for Determination of Glutamate Based on [4](1)(2,3-Diazabuta-1,3- diene) ferrocenophane

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A novel potentiometric membrane sensor has been developed and optimized based on [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane as an ionophore in poly(vinyl chloride) matrix membrane (PVC) plasticized with tris(2-ethylhexyl) phosphate (TEHP) for high selective determination of glutamate. The sensor works well in a linear range of $1.0x10^{-5}$ to $1.0x10^{-1}$ M glutamate with a Nernstian slope of 57.6±1.0 mV/decade and its detection limit is $7.95x10^{-6}$ M. The sensor shows working range over the pH 6–10 at temperature 25±1 °C and stable for a period of 3 months without any divergence in potentials with response time ≤30s. The selectivity coefficient values as determined by mixed interference method indicate a good selectivity for ions over a wide variety of other tested anions.

Keywords: [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane; Glutamate membrane sensor; Monosodium glutamate; Potentiometric

1. INTRODUCTION

Glutamate is an important neurotransmitter in the mammalian central nervous system and neuronal pathways in the brain. It is one of the 20 standard amino acids used by all organisms which play an important role in food processing and also in clinical applications. Its monosodium salt (monosodium glutamate, MSG), well known as a flavor enhancer is widely used thus give an enjoyable taste known as Umami. The excessive intake of this flavor enhancer causes excessive stimulation of glutamate receptors in the central nervous system of vertebrates as well as glutamate release from neurons which lead to neuronal degeneration and cell death thus contribute to several

neurological disorders including stroke, epilepsy, schizophrenia, Alzheimer's disease and Parkinson's disease [1-3] as well as to memory and learning processes [4,5]. Therefore there is a need to determine its presence in a variety of food samples.

A [4](1)(2,3- Diazabuta-1,3-diene)ferrocenophane (Figure 1) is an example of many ferrocene derivatives. It is centrosymmetric and the cyclopentadienyl rings of the ferrocene moieties are eclipsed and their average dihedral angle with the Schiff base C=N−N=C fragment is 8.5 (4)° [6]. It is a type of 'organo-iron compound' which is aromatic, highly stable and soluble in most common organic solvents [6]. An iron metal was sandwiched by two cyclopentadienyl (Cp) ligands which lie in parallel planes. The Cp ligands are bonded covalently to the iron center [7]. Ferrocene derivatives are highly thermally stable, withstanding temperatures as high as 500°C, stable in air, so that they can be used in a wide variety of application without fear of degradation. Ferrocene group is particularly attractive electrochemical agents for use in chemical sensing and other technologies [8]. Example, strained [1]ferrocenophanes and [2]ferrocenophanes were used as monomers (to make up as polymers with metals) to incorporate in the backbone for particular interest [9,10].

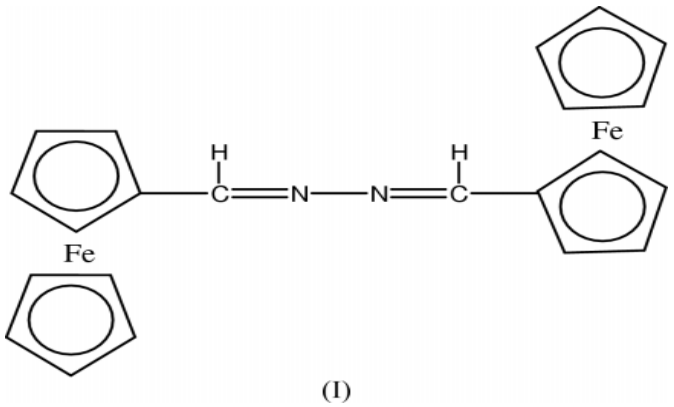

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Figure 1. Molecular structure of [4](1)(2,3- Diazabuta-1,3-diene)ferrocenophane.

Numerous analytical methods for determination of glutamate have been developed using different detection techniques such as chromatography [11-15], spectrophotometric [16-18], fluorimetric [19-22], amperometric [23-27] but they are considered to be time consuming, having a long response time, high cost and the analyte needs to be prepared into simpler form before some techniques could be applied. The alternative is ion-selective electrode (ISE) which offers speed, fast response, simplicity, low cost and wide concentration range [28]. ISE is a useful tool for the potentiometric measurement of the activity of an ion in the presence of other ions. A lot of work has been done on ISEs in determination of cations [29-42], and anions [43-52] but there are few reports of selective electrodes for glutamate determination [53,54]. In this study, a novel potentiometric sensor for selective determination of glutamate was introduced with [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane as the ionophore in PVC matrix.

2. EXPERIMENTAL

2.1. Reagents

All reagents used for the preparation of membranes were of analytical-reagent grade and were used without further purification. High molecular weight poly(vinyl chloride) (PVC), obtained from Fluka Chemika (Switzerland). Tris(2-ethylhexyl)phosphate (TEHP), 2-nitrophenyloctylether (2-NPOE), sodium salts of all anions and tetrahydrofuran (THF) were obtained from Merck (Germany). Plasticizer diocthyl phthalate (DOP), Britton-Robinson buffer and sodium hydroxide solution for pH adjustments and ferrocenecarboxaldehyde 98% were obtained from Aldrich (Germany). Stock solutions of glutamate were freshly prepared by dissolving appropriate amount of monosodium glutamate in distilled deionised water.

2.2. Instrumentation

All solutions were prepared using distilled deionised water from EASYpure LF, Barnstead (USA). The potentiometric measurements were performed using an Orion 720A, Mass. (USA) pH meter. An Ag/AgCl of BAS (UK) was used as a reference electrode. The pH value was determined by using Orion, 915600, Mass. (USA) glass-pH electrode. All electromotive force (emf) measurements were carried out with the following cells assemblies:

Ag, AgCI; KCl(satd.)// sample solution/membrane/glutamate solution(sat.)/Pt wire

2.3. Preparation of Membrane Electrode

The membrane electrode was prepared by thoroughly dissolving amount of PVC, plasticizers and ionophore in 5 ml THF. The ionophore, [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane was prepared according to a previously published paper [6]. The mixture was vigorously stirred immediately after the addition of THF for an easy dissolution of PVC. After complete dissolution of all components, the homogeneous mixture obtained was then poured in a glass ring with an inner diameter of 3.5 cm, which sat on a surface of glass plate. A filter paper was placed on top of the glass ring to prevent dust and air streams from spoiling the mixture. The mixture was then allowed to evaporate at room temperature. After 24 hours, the membrane was later peeled off from the glass. A membrane disc of 6 mm diameter was then cut and glued to one end of a Pyrex glass tube. The glass tube was then filled with an internal solution of saturated glutamate solution and electrical contact was done by immersing a platinum wire in the solution. The sensor was conditioned for 24 hours by soaking in 1.0 x 10^{-5} M glutamate solution and rinsed well with distilled water and stored in 1.0 x 10^{-5} M glutamate solution when not in use.

The ratio of membrane ingredients, time of contact and concentration of equilibrating solution were optimized to provide membranes which develop reproducible, stable and noiseless potentials [55-57]. The potentiometric response refers to the ion exchange mechanism at the membrane-solution

interface. The possible mechanism of glutamate and [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane that is responsible for the potentiometric response is shown in Figure 2.

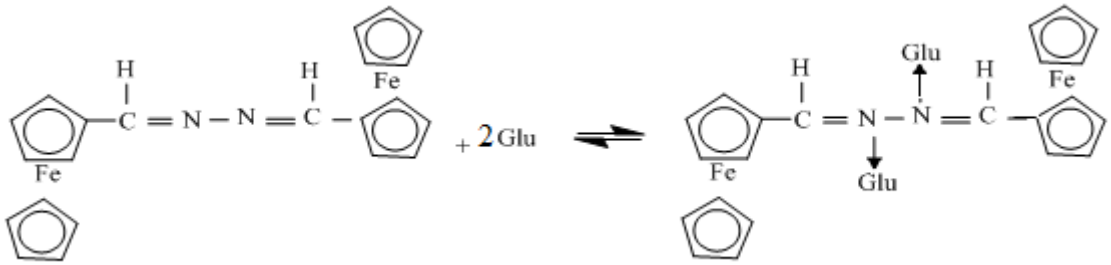

Figure 2. Possible mechanism at the membrane-solution interface.

2.4. Measurement of glutamate in food samples

All food samples (oyster sauce, seasoned flour and prawn snack) were obtained from local supermarkets. About 1 g of each food samples was added into 50 mL of water to be heated about 70 ^{o}C for 10min to discard any available fat [58]. Then, the sample was cooled to room temperature and was then filtered through Whatman filter paper No. 1. The supernatant was then diluted to 1000 mL in volumetric flask before undergo the emf measurements.

The measurements were done by immersing the proposed electrode and the reference electrode into 50 mL solution of sample. The solution sample was continuously stirred during measurement and the measurements were recorded when the potential reading was stable. The results obtained were compared with the standard method for glutamate determination.

3. RESULTS AND DISCUSSION

3.1. Sensors Characteristics

Several membranes of different compositions were prepared and investigated. Sensors with membranes incorporating [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane in plasticized poly(vinyl chloride) in the ratio of 10 wt% ionophore, 36 wt% PVC and 54 wt% plasticizer were prepared. Different plasticizers (2-NPOE, DOP and TEHP) were added to investigate the performance of glutamate membrane sensor. The influence of membrane composition on the potentiometric responses of glutamate membrane sensor based on [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane was summarized in Table 1. The working concentration range, calibration slope and selectivity of a membrane sensor are significantly depending on the membrane composition and the nature of plasticizer used [55,59,60].

Table 1. Optimized membrane composition of glutamate membrane sensor based on [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane and their potentiometric responses.

Membrane electrode	Ionophore (mg)	PVC (mg)	Plasticizer (mg)	Additive (mg)	Working concentration range (M)	Slope (mV/ decade)	Detection limit (M)
1	10	40	-	-	$1.0x10^{-1}$ - $1.0x10^{-4}$	51.3	6.30×10^{-5}
2	10	40	60 (2-NPOE)	-	$1.0x10^{-1}$ - $1.0x10^{-4}$	46.1	1.51×10^{-4}
3	10	40	60(DOP)	-	$1.0x10^{-1}$ - $1.0x10^{-4}$	53.9	4.27×10^{-5}
4	10	40	60(TEHP)	-	$1.0x10^{-1}$ - $1.0x10^{-5}$	57.6	7.95×10^{-6}

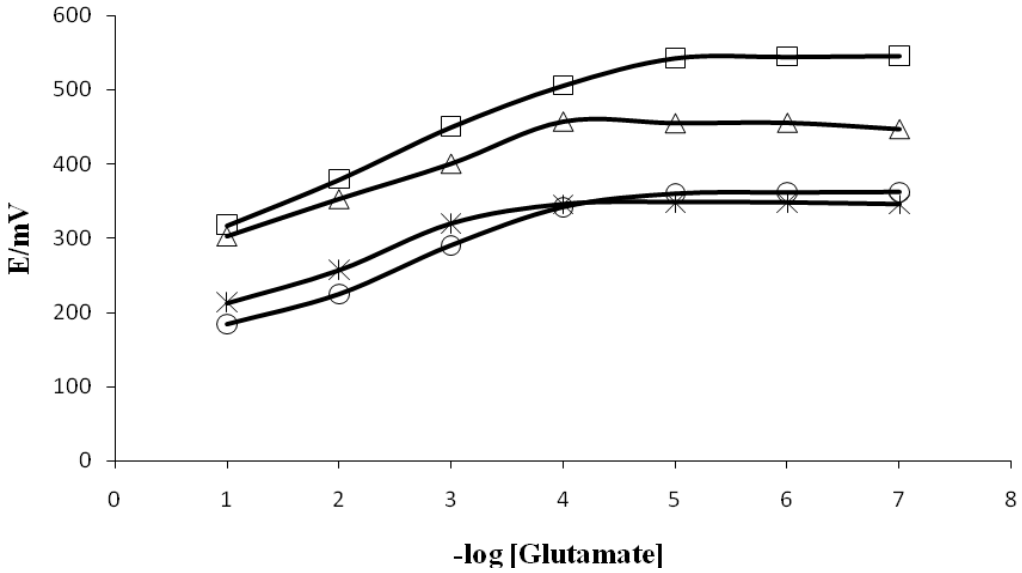

Figure 3. Potential response of glutamate membrane sensor based on [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane at different membrane composition. (△ **:** membrane No.1, * **:** membrane No.2, ○**:** membrane No.3, □ membrane No.4).

Data presented in Figure 3 and Table 1 showed that the glutamate membrane sensor based on [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane (sensor no.1, without plasticizer) exhibited working range 1.0×10^{-1} – 1.0×10^{-4} M glutamate solution with a calibration slope of 51.3 mV decade^{-1} and detection limit is 6.30×10^{-5} M. Next, the addition of plasticizers 2-NPOE and DOP (membrane no. 2 and 3) both showed linearity over the working concentration range 1.0×10^{-1} – 1.0×10^{-4} M glutamate solution with weak calibration slope of 46.1 mV decade^{-1} and 53.9 mV decade^{-1} and detection limit 1.51×10^{-4} M and 4.27×10^{-5} M respectively. However the addition of TEHP (membrane no. 4) improved the working concentration range of 1.0×10^{-1} – 1.0×10^{-5} M glutamate solution with Nernstian slope 57.6 mV decade^{-1} and detection limit is 7.95×10^{-6} M. The standard deviation of 20 identical measurements is ± 0.2 mV. It seems that TEHP improves the membrane potentiometric responses due to the interaction of glutamate ions and the membrane and thus enhances the complex

formation. Optimal potentiometric responses were obtained by using saturated glutamate as an internal filling solution. The potentiometric responses of [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane based membrane sensor supports the mechanism and complex structure showed in Figure 2, where lone pairs from a nitrogen atom in [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane form dative covalent bond to a glutamate. This is a reversible reaction. This analogy taken from the formation of the ammonium ion. The potentiometric responses are attributed to the ion exchange mechanism at the membrane-solution interface because of its univalent charge of glutamate and [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane behaves as a charge carrier for glutamate [53]. In comparison, the proposed glutamate membrane sensor based on [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane possesses wide working concentration range which equal or better than other techniques for glutamate determination [Table 2].

Table 2. Comparison between the proposed glutamate membrane sensor based on [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane and other reported techniques.

Technique	Working concentration range (M)	References
[4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane membrane sensor	1.0×10^{-5} - 1.0×10^{-1}	This work
Chitosan membrane sensor	1.0×10^{-5} - 1.0×10^{-1}	53
Enzyme fluorometric	1.0×10^{-5} - 5.0×10^{-4}	61
Enzyme amperometric	5.0×10^{-7} - 6.0×10^{-5}	62

Hence, membrane sensor no. 4 was selected as glutamate ion selective electrode (ISE) and all further investigations were carried out with this membrane sensor.

3.2. Response Time and Lifetime

The response time of [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane based membrane sensor for glutamate determination was determined by immerse the sensor in 1.0×10^{-7} – 1.0×10^{-1} M glutamate solutions. The time required to achieve a steady potential is within 30s for solutions of concentration $\leq 1.0 \times 10^{-5}$ M and 15s for solutions with concentration $\geq 1.0 \times 10^{-4}$ M. The response time is more rapid when proceeding from diluted to concentrated solutions. The membrane was stored in 1.0×10^{-5} M glutamate solution when not in use.

The dynamic response is plotted as emf against time and is shown in Fig. 4. The time needed to reach a potential within ±1 mV of the final equilibrium value after successive immersion of a series of glutamate solutions, each having a 10-fold difference in concentration is 15s.

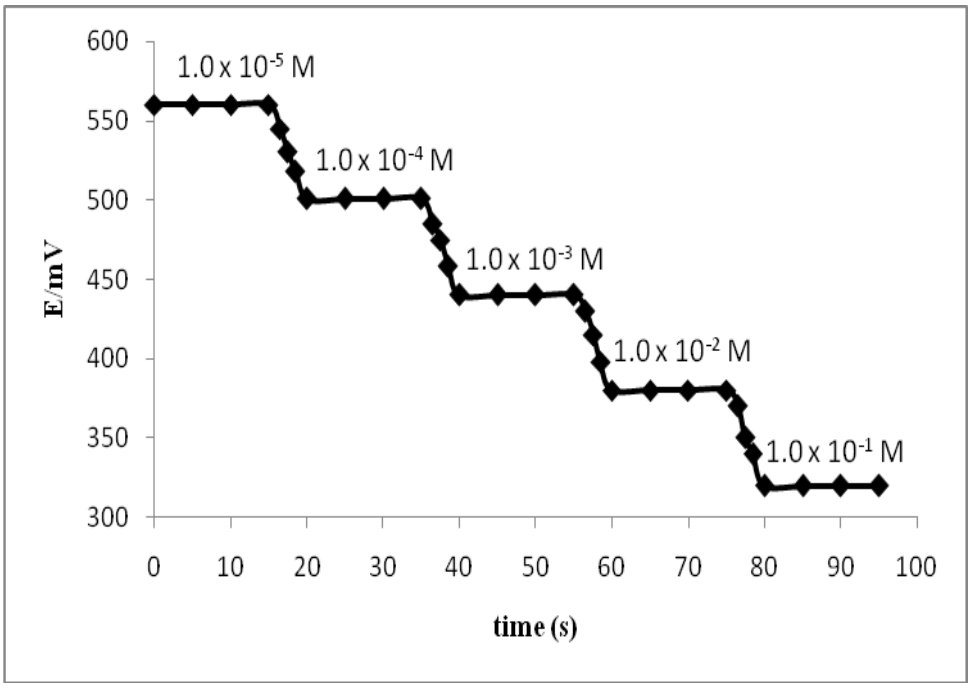

Figure 4. The dynamic response study of membrane electrode No. 4

Lifetime of a membrane based sensor depends on the distribution of an ionophore and plasticizer between the analyte solution and membrane phases. The lifetime of [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane based membrane sensor for glutamate determination is about 3 months. The values of detection limit and calibration slope were fairly stable during this time.

3.3. pH Effect

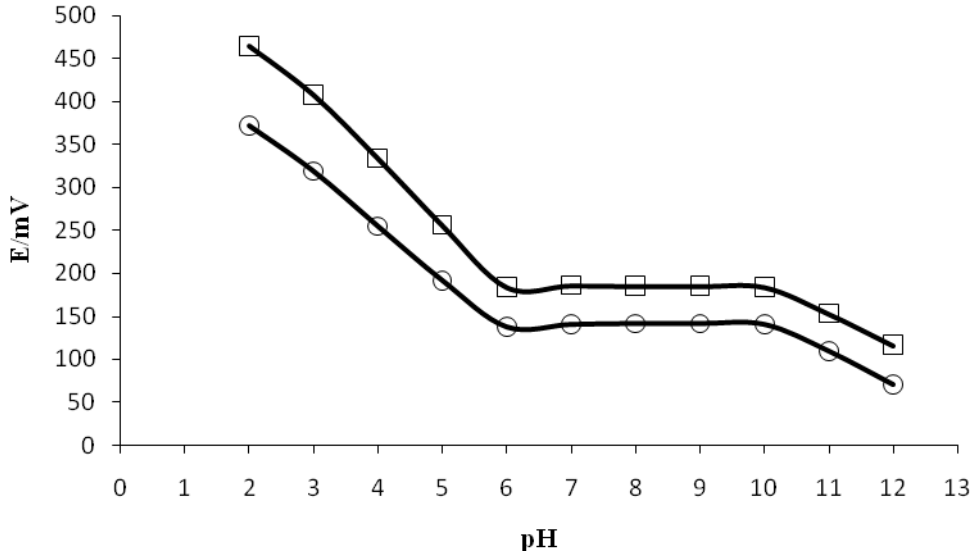

Figure 5. Effect of pH at (□) 1.0 x 10^{-3} M and (○) 1.0 x 10^{-4} M glutamate solutions on the potential response of membrane No. 4.

The potential response of [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane based membrane sensor was sensitive to pH changes and had been tested over the pH range from 2 to 12 for different concentration of glutamate solutions (1.0×10^{-3} and 1.0×10^{-4} M). The pH of glutamate solutions was adjusted with Britton-Robinson buffer solution and 0.2 M sodium hydroxide solution. The results showed that the potential is pH independent in the range of pH 6-10 (Fig. 5). There was a sharp change in the potential response at higher and lower pH values due to the interaction of the membrane components with the analyte ions. In more acidic condition, glutamate is protonated to glutamic acid thus distract the ability of a membrane to interact with glutamate ions in solution. On the other hand, changes of potential probably due to the interference of hydroxyl ions which compete for the cationic site in the membrane in more basic solution [53].

3.4. Potentiometric Selectivity Coefficient

Potentiometric selectivity coefficient (K^{pot}) has been described in terms of relative electrode response for the primary ion over the other ions present in the solution. This is the most important characteristic as it determines the utility of a sensor in real sample measurement.

Table 3. Selectivity coefficients of glutamate membrane sensor based on [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane towards various interfering ions.

Interfering ion	Kpot Membrane electrode No.4
I^-	1.0×10^{-2}
Br^-	1.2×10^{-2}
Cl^-	1.5×10^{-2}
OH^-	1.0×10^{-2}
NO_3^-	1.0×10^{-2}
$CH3COO^-$	1.0×10^{-2}
SO_3^{2-}	2.5×10^{-2}
SO_4^{2-}	1.0×10^{-2}
CO_3^{2-}	1.5×10^{-2}
CrO_4^{2-}	4.0×10^{-2}
CrO_7^{2-}	1.2×10^{-2}
HPO_4^{2-}	1.0×10^{-2}
PO_4^{3-}	1.2×10^{-2}
Citric acid	7.9×10^{-3}
Aspartic acid	6.3×10^{-3}
Glycine	1.5×10^{-2}

Mixed solution method was selected as it is similar to real sample condition. Values of K^{pot} for the proposed sensor was measured by mixing a series of interfering anionic solution (1.0×10^{-1} - 1.0×10^{-7} M) with 1.0×10^{-3} M glutamate solution prepared at pH 10. The results are given in Table 3. From Table 3, it can be seen that [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane based membrane sensor is

selective towards glutamate with respect to monovalent, divalent and trivalent anions. In addition, the K^{pot} values were much less than 1.0.

The interfering effect of the ions is in the following order:

NO_3^- = SO_4^{2-} = CH_3COO^- = I^- = OH^- = HPO_4^{2-} > PO_4^{3-} = CrO_7^{2-} = Br^- > Cl^- = CO_3^{2-} = glycine > SO_3^{2-} > CrO_4^{2-} > aspartic acid > citric acid.

The selectivity pattern for glutamate based on [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane membrane sensor shows a deviation from Hofmeister series, which R^- > ClO_4^- > SCN^- > I^- > NO^{3-} > Cl^- > HCO_3^- > SO_4^{2-} > HPO_4^{2-}. The deviation results from the unique interactions between ionophore and anions. In conclusion, the proposed sensor would not cause interference towards the studied interfering ions in the determination of glutamate.

3.5. Analytical Applications

The proposed sensor was applied in the determination of glutamate in food samples (oyster sauce, seasoned flour and prawn snack). Table 4 shows the results obtained by the proposed membrane sensor compare favorably with the liquid chromatography method. The amount of glutamate present in the food samples is acceptable by the Foods Act and Food Regulation [63].

Table 4. Analysis of glutamate in food samples by the proposed membrane electrode and the liquid chromatography method.

Food samples	Glutamate (%)*	
	Proposed membrane electrode	HPLC
Oyster sauce	4.0 ± 0.2	3.9 ±0.3
Seasoned flour	6.4 ± 0.3	6.1 ± 0.2
Prawn snack	10.1 ± 0.2	10.3 ± 0.3

* All the values were the mean of triplicate measurements (n = 3).

4. CONCLUSIONS

A simple, fast response, low cost and wide concentration range of glutamate ion-selective electrode has been developed based on [4](1)(2,3-Diazabuta-1,3-diene)ferrocenophane. The sensor exhibits good reproducibility over a period of 3 months and performs high sensitivity, stability, response time and detection limit. It is easily to carry out in any laboratory equipped with pH ionmeter. High selectivity of the proposed sensor made it possible to determine the presence of glutamate in any food samples without converting the sample to more volatile analyte.

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