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Determination of Glutamate in Food Samples by Heterogeneous Membrane with Chitosan as Ionophore

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Abstract

This study reported on glutamate sensors based on chitosan as a heterogeneous membrane. The linearity was found to vary from 1.0×10^{-5} M to 1.0×10^{-1} M. The limit of detection was 5.0×10^{-6} M and the pH values were in the range from 4 to 6. The presence of ionic species normally found in foodstuffs did not interfere the electrode. The material for proposed electrodes were cheap and environmental friendly and therefore are suggested as alternative tools for the analysis of glutamate.

Keywords: Chitosan, heterogeneous membrane, glutamate

Abstrak

Kajian ini melaporkan pengesan glutamat berdasarkan kitosan sebagai membran heterogenus. Julat linernya ialah antara 1.0×10^{-5} M hingga 1.0×10^{-1} M. Had pengesanannya ialah 5.0×10^{-6} M dan nilai pH ialah antara 4 hingga 8. Kehadiran spesies berion yang biasa didapati di dalam makanan tidak mengganggu elektrod tersebut. Bahan elektrod tersebut murah dan mesra alam dan oleh itu ia boleh dicadangkan sebagai alat alternatif untuk analisis glutamat.

Kata kunci: Kitosan, membran heterogenus, glutamat

Introduction

Monosodium glutamate (MSG) also synonymous as Ajinomoto, is the salt of one of the non-essential amino acid, glutamic acid – most abundant amino acids found in nature. The MSG has a unique taste called umami which is quite distinct from the other four basic tastes (sweet, sour, salty and bitter). It has been widely used as flavor enhancer (E621) and also as additive in food ever since first invented by Kikunae Ikeda in 1907. But, the MSG has also been blamed to have caused a range of adverse effects to those eating foods containing large amount of this compound. Such complex

symptoms like dizziness, headache, numbness, chest pain, sweat and even glaucoma, popularly known as Chinese Restaurant Syndrome which are common to those effected. For this reason, food act has been stipulated on the quality as well as quantity of MSG used (Malaysian Food Acts, 1983). In the UK, it is reported (Rhodes et al.,1991) the average intake of MSG is 590 mg/day, with extreme users consuming 2330 mg/day. In a highly seasoned meal, the average intake reaches as high as 5000 mg/day or even more (Yang et al.,1997). However no lethal dosage has been stated by any authority on the intake of MSG. Even Federal Drug Authority (FDA) classifies MSG as generally recognized as safe (GRAS). There is no epidemic, as yet, but the rampant use of MSG especially, in fast food chains is, indeed, alarming. As the MSG scare is imminent the public nowadays are taking "better safe than sorry" attitude i.e. by choosing foods with "MSG free" label.

Many analytical methods are used for the analysis of glutamate. The most popular, is by liquid chromatography (LC) (Hanko & Rohrer, 2004; Lu et al., 2005, Qu et al., 2002). However, this technique is relatively tedious. The electrochemical methods are also used (Arkady et al., 2000; Khampa et al., 2004; Md et al., 2005; Nobutoshi et al., 2002; Weite et al., 2006). But they are mostly classified as enzyme amperometric technique. Other methods of choice are spectrophotometric (Chapman & Zhou, 1999; Fonda, 1985; Lee et al., 1999) and capillary electrophoresis (Chanda & Michael, 2007; Mark et al., 1997).

Chitosan, a poly-[1-4]- β-*D*- glucosamine, is a derivative of chitin – a naturally occurring polysaccharide usually found in crustaceans. It is a natural chelate, commonly used for the complex formation with transition metals ions (Wan Ngah & Isa, 1997; Riccardo et al., 1974). It is reported capable of removing proteins from water (Gamage & Shahidi, 2007). The recovery of proteinaceous materials present in food processing operation is also reported (Knorr, 1991).

In this study, the performance of potentiometric sensor based on heterogeneous chitosan membrane for the determination of glutamate are described as in the following.

Experimental

Chemicals and Reagents

The chitosan powder (granular, 100 mesh) PM 100 was obtained from Chito Chem. (Malaysia) and was used without purification. Analytical grade sodium salt of L-glutamate acid (MSG), lycine and glycine were obtained from BDH chemicals (England). High-molecular weight poly(vinyl chloride) (PVC), 2-nitrophenyloctylether (2-NPOE), bis(2-ethylhexyl)adipate) (BEHA) and diocthyl phenylphosphonate (DOPP), aspartic acid, ascorbic acid, citric acid and sodium benzoate were obtained from Fluka Chemika (Switzerland). Analytical grade potassium and sodium salts of

all anions and tetrahydrofuran (THF) were obtained from MERCK (Germany). All other reagents and chemicals were of analytical grade reagent.

All solutions were prepared using pure water from Milli–Q Plus, Millipore Corp. (USA). The pH adjustments were made with 1.0 M hydrochloric acid and 1.0 M sodium hydroxide solution. Stock solution of 1.0 M MSG was freshly prepared everyday prior to experiment.

Instrumentation

Potential measurements were carried out with pH ion meter Orion 720A, of Orion Inc. (USA). A saturated calomel electrode of Russell (UK) with a fiber junction was used as a reference electrode. The pH value was determined by using combination glass electrode type Orion 915600, of Orion Inc. (USA). The IR spectra were recorded by using FTIR spectrometer, model Spectrum BX of Perkin–Elmer (USA).

Preparation of Heterogeneous Membrane

The chitosan of various ratio was mixed with a fixed ratio of PVC (initially dissolved in 2 mL tetrahydrofuran (THF)) to get the optimum performance. The best membrane was then blended with 10 drops of plasticizers. The blend was poured into a glass ring (3.5 cm i.d.) on a smooth glass plate and allowed to stand at room temperature for 24 hours. A heterogeneous membrane of 6 mm in diameter was cut out and then attached to one end of a Pyrex® glass tube with Araldite®. The glass tube was then filled with saturated sodium salt of L-glutamic acid prior to conditioning in the 1.0×10^{-5} M of sodium salt of L-glutamic acid. The cell scheme for heterogeneous membrane was as in the following;

$$Pt|glutamate(sat)|membrane|sample||KCI(sat)|Hg_2CI_2|Hg \quad (i)$$

Characterization of Membranes

The membrane was first conditioned by equilibrating in 1 M sodium chloride at pH 5.8 ± 0.7 for 24 hours. Drop wise addition of sodium acetate was used to neutralize the acid present. The physicochemical properties of the membrane were determined by adopting methods as described elsewhere (Amarchand et al., 2000; Khan & Inamuddin, 2006).

Water Content (% total wet weight)

The conditioned membrane was first soaked in water to elute diffusible salts and then blotted quickly with Whatman filter paper to remove surface moisture, and

immediately after, it was weighed. It was further dried to a constant weight in a vacuum desiccator over phosphorus pentoxide for 24 hours. The water content (% total wet weight) was calculated as:

$$\% \text{ Total wet weight} = \frac{W_w - W_d}{W_w} \times 100 \quad \text{----------(ii)}$$

where W_w was the weight of the soaked/wet membrane and W_d was the weight of the dry membrane.

Porosity

Porosity (ε) was determined as the volume of water incorporated in the cavities per unit membrane volume from the water content data.

$$\varepsilon = \frac{W_w - W_d}{AL\,\rho_w} \quad \text{----------(iii)}$$

where W_w and W_d were the weights of wet and dry membranes respectively. A and L were the area and thickness of the membrane, and ρ_w was the density of water.

Thickness and Swelling

The thickness of the membrane was measured by taking the average thickness using digital screw gauge. Swelling is measured by the difference between the average thickness of the membrane equilibrated with 1 M NaCl for 24 hours and the dry membrane.

Characterization of Adsorbent

The membrane was first immersed in saturated glutamate solution at pH 6 for 24 hours and then was flushed with pure water. The FTIR spectrum of the membrane was recorded in the range of 400–4000 cm^{-1}.

Measurement of Glutamate Response

A series of standard solutions in the range of 1.0×10^{-7} to 1.0×10^{-1} M glutamate were prepared for calibration plot. The selectivity of both electrodes were done in mixed solution manner. The potential response was measured from mixture of interfering ion solution (1.0×10^{-7} – 1.0×10^{-1} M) and 1.0×10^{-4} M glutamate. For real sample analysis, solid samples have been discarded of any available fat. This was done by dissolving the accurately weighed solid in a 50 mL of deionized distilled water and then heated at about 70 °C for 10 min, and then cooled to room temperature before

filtering through Whatman no. 1 (Khampa et al., 2004). The supernatant samples were further diluted with distilled deionized water.

Result and Discussion

Four heterogeneous membranes with chitosan : PVC (%w/w) ratio of 0.25 : 1 , 0.5 : 1, 0.75 : 1, 1 : 1, 1 : 0.25, 1 : 0.5 and 1 : 0.75 have been studied. The 1:1 ratio membrane is chosen as the best. It is noted that with the addition of plasticizers the physical characteristics of membranes are altered (Table 1). It seems that all the membranes are of less porosity. But the swelling increases in the order of : without plasticizer > DOPP > BEHA > 2–NPOE . The low orders of porosity as well as H_2O content, swelling and with less thickness suggest that interstices are negligible, especially with membrane without plasticizer. This infers that diffusion across the membrane have occurred mainly through the exchanges sites (Khan & Inamuddin,2006). Thus, membrane without plasticizer is used for the final preparation of the ion selective electrode (ISE) assembly for further studies.

The electrode response is the result of exchange mechanism between the glutamate ion in solution and those in the membrane at the protonated amine group as in equation (v),

$$\underset{\text{(membrane)}}{\text{Chitosan–NH}_3^+ – \text{Glu}^-} + \underset{\text{(solution)}}{\underline{Glu}^-} \rightleftarrows \underset{\text{(membrane)}}{\text{Chitosan–NH}_3^+ – \underline{Glu}^-} + \underset{\text{(solution)}}{\text{Glu}^-} \text{ ------(v)}$$

The infrared vibrational peaks of chitosan and glutamate–chitosan membranes are shown in Figure 1. The spectra display a number of absorption peaks, which indicates the complex nature of the adsorbent. The strong absorption bands ranging from 3000 to 3600 cm^{-1} in glutamate–chitosan membrane indicates the presence of amino acid group caused by the H–bonded NH_2 and OH stretching (Joseph et al., 1998). The Donnan equilibrium is achieved when no more exchange mechanism occurs between the membrane and the solution. The response time of 20–35 s is the time taken for the electrode's reading to become constant. The extrapolation of the linear range of the standards calibration plot (Figure 2) to the base line produces limit of detection (LOD) at 5.0×10^{-6} M , the working range between 1.0×10^{-5} to 1.0×10^{-1} M and optimum pH 6. A typical slope of 53 ± 1 mV decade $^{-1}$ is obtained. Figure 3 indicates that the electrode potential remains unchanged within a pH range of 4 – 8. Above pH 8, the electrode behaves in an erratic manner, due to interference of hydroxyl ion competing for the anion site in the membrane. A sharp decrease in pH value below 4.0 is due to protonation of glutamate to undissociated glutamic acid. The selectivity coefficient (K^{pot}) values in Table 2 indicate that the heterogeneous membrane type is relatively selective towards glutamate ion over several anions tested. The lifetime of the heterogeneous membrane lasted for at least 4 months. The result indicates that a

longer life and stable electrode has been obtained. The electrodes compare favorably if not better than some of the earlier reported (Hanko & Rohrer, 2004; Ling et al., 2000; Stalikas et al., 1993).

The performance of heterogeneous membrane is shown in Table 3. Table 4 shows the results obtained in this study compare favorably with that of others reported (Khampa et al., 2004; Oliveira et al.,2001; Lau & Mok, 1995).

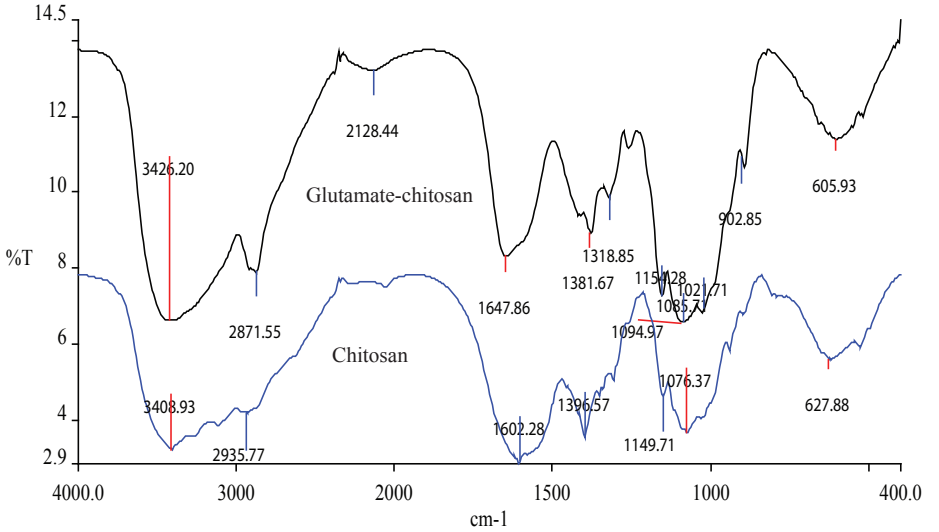

Figure 1 FTIR spectrum of heterogeneous membrane before and after reaction with glutamate

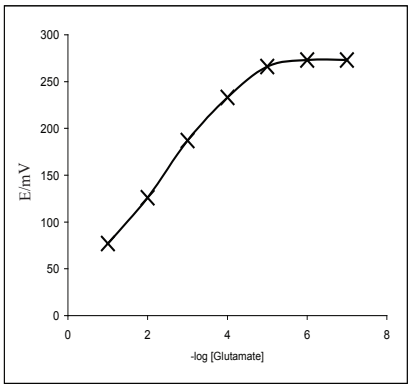

Figure 2 The e.m.f response of the optimized heterogeneous membrane without plastisizer in standard glutamate solutions

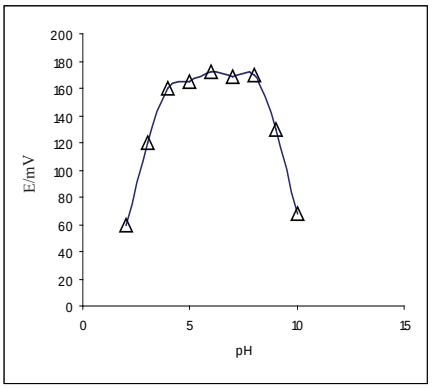

Figure 3 Effect of pH on heterogeneous membrane without plasticizer in 1.0×10^{-3} M glutamate

Table 1 The characterization of plastisized heterogeneous membranes type

Membrane with and without plasticizer	Thickness (mm)	Water content (% weight wet	Porosity	Swelling (% weight wet)
-	0.137	13.04	0.0447	0.44
DOPP	0.153	9.30	0.0533	1.48
BEHA	0.150	13.64	0.0713	2.53
2-NPOE	0.143	12.03	0.0678	4.79

Table 2 Selectivity coefficients of heterogeneous membrane type towards various interfering ions

Interfering	K^{pot}
CI^-	3.55×10^{-3}
Br^-	2.51×10^{-3}
NO_3^-	2.24×10^{-3}
SO_4^{2-}	2.51×10^{-3}
$C_2O_4^{2-}$	5.51×10^{-3}
PO_4^{3-}	5.62×10^{-3}
PO43-	2.51×10^{-3}
Ascorbate	5.62×10^{-3}

Table 3 Performances of heterogeneous membrane

Proposed glutamate ISE	Response time (s)	Shelf life (month)	pH		Linear range (M)	LOD (M)
			range	optimum		
Heterogeneous membrane	20 – 35	4	4 – 8	6	1.0×10^{-5} to 1.0×10^{-1}	5.0×10^{-6}

Table 4 Glutamate assay by the proposed and literature

	Linear range	LOD
Isa et al.	10 μM - 1 mM	10 μM
Khampa et al.	0.2 - 20 μM	0.14 μM
Oliveira et al.	2.5 - 75 mM	-
Lau et al.	0 - 500 μg	0.007% (w/w)

Table 5 Real sample analysis of MSG

Samples	MSG (% w/w)	
	Heterogeneous membrane	HPLC
Campbell	0.25 ± 0.01	0.36 ± 0.01
Prego	0.44 ± 0.01	0.53 ± 0.01
McCormick	3.11 ± 0.05	2.94 ± 0.04
Local brand 1	14.2 ± 0.3	16.6 ± 0.3
Local brand 2	15.6 ± 0.2	14.4 ± 0.2
Local brand 3	16.4 ± 0.2	15.9 ± 0.3

All the values were the mean of triplicate measurements in percentage by mass

The proposed heterogeneous membrane electrode are successfully applied for the analysis of MSG in food samples. The results for the analysis of MSG by the developed sensors and their validations are summarized in Table 5. The data obtained by the proposed sensors compare favorably with the liquid chromatography method (Jan, 1998). Good correlation was obtained between the proposed and the standard method (HPLC), i.e. 0.977. It is then concluded the proposed sensors can be successfully used for the selective determination of MSG in food.

Conclusions

Glutamate ISE based on chitosan as heterogeneous membrane has been developed. The strength of the proposed method was its simplicity, specificity, sensitivity and non-toxic as opposed to the more exotic methods mentioned in the article. The measurement was comparable to the later. The proposed method also exhibit favorable performances on their pH, limit of detection, calibration slope and response. The chitosan used was a non – toxic and biodegradable material. This would then reduce the environmental impact had hazardous chemical was used in electrode fabrication.

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