Food Chemistry 112 (2009) 756–759

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

A non-plasticized chitosan based solid state electrode for flow injection analysis of glutamate in food samples

Illyas Md Isa, Sulaiman Ab Ghani *

Pusat Pengajian Sains Kimia, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia

ARTICLE INFO

Article history:
Received 23 May 2007
Received in revised form 18 June 2008
Accepted 21 June 2008

Keywords:
Flow injection analysis
Glutamate
Graphite pencil rod
Non–plasticized
Solid state electrode

ABSTRACT

This report describes the development of solid state glutamate sensor based on chitosan. The electrode has linearity range of 0.01–1 mM glutamate with detection limit of 0.008 mM. The presence of other conventional food additives at physiological level does not interfere. The interfering effect is, however, minimized through prior dilution of the sample. Recovery values of 88.9–99.2% are obtained throughout. The proposed electrode has been applied in flow injection analysis (FIA) glutamate in food samples. In the validation experiment, the proposed electrode is found to be comparable with the standard method.

1. Introduction

Glutamic acid is the most studied amino acid. It is mostly found in sea creatures, especially, fish. Hence, it is an indicator for fish freshness. But glutamic acid is not a stable compound as it deteriorates fast in air. Its stable salt, monosodium glutamate (MSG), however, is widely used as a food flavor enhancer and also as an additive in processed, canned and packed foods. The good taste of the food added with MSG has often caused people to consume higher dosage of this compound. Any excess of MSG in our body could interrupt the balance of body glutamate, a neurotransmitter, by over stimulating brain functions. This will then produces symptom such as dizziness, headache, numbness, chest pain and sweat. Such symptom is, popularly, known as 'Chinese Restaurant Syndrome', named after an outbreak of incidence in New York Chinese Restaurants in 1968 (<http://www.nlm.nih.gov/medlineplus/ency/article/001126.htm>).

Chitosan, a natural polymer, is good in uptaking transition metals (Riccardo, Muzzarelli, & Rochetti, 1974). This is, realized, via coordination complex formation at the secondary amine functional group within the polymer unit. It is, commonly, known that in cosmetic industry, chitosan is used as an ingredient for slimming (fat remover) purposes. However, there has been no report thus far on the uptake of fat, i.e. carboxylic acid, by chitosan. Except that chitosan is reported capable of up taking proteins (Inoue, Baba, Yashizuka, Noguchi, & Yoshizaki, 1988; No, 1987; Senstad & Almas, 1986), and the reaction depends on pH (No, 1987) as well as protein – chitosan ratio (Senstad & Almas, 1986).

Various instrumental methods have been developed for the analysis of glutamate. But the most popular analysis by far is liquid chromatographic (LC) methods (Halmos et al., 2002; Hanko & Rohrer, 2004; Lu, Chiu, Chang, Ho, & Chang, 2005; Qu et al., 2002; Tcherkas & Denisenko, 2001) and electrochemical methods (Chang, Hsu, Chen, Chang, & Chen, 2003; Chapman & Zhou, 1999; Khampa, Meevootisom, & Wiyakrutta, 2004; Ling, Wu, Wang, Wang, & Song, 2000; Niwa, Horiuchi, Kurita, Tabei, & Torimitsu, 1998; Oliveira et al., 2001; Ryan, Lowry, & O'Neill, 1997). In LC method, glutamate, prior to injection into column, has to be derivatized with *o*-phthalaldehyde in the presence of 2-mercaptoethanol (Van Hemelrijck, Sarre, Smolders, & Michotte, 2005). It has been reported (Tcherkas & Denisenko, 2001; Bogdanov, Tjurmina, & Wurtman, 1996) that glutamate does not derivative easily. The separated glutamate is then detected by enzyme-based amperometric sensors (Chang et al., 2003; Chapman & Zhou, 1999; Khampa et al., 2004; Oliveira et al., 2001). It is obvious that LC analysis of glutamate acid is not cheap and also time consuming. Hence, there is a need to find alternative for such analysis.

The potentiometric analysis using solid state electrode and its advantage has been described (Cattrall, Drew, & Hamilton, 1975). This could be the appropriate option for the standard method in glutamate determination. The solid state electrode based on chitosan has been used in the potentiometric analysis of iron (III) (Guanghau, Xin, Xiaogang, & Tong, 2001).

* Corresponding author. Fax: +6 04 6574854.
E-mail address: sag@usm.my (S. Ab Ghani).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.06.043

In this report we aim to develop the non-plasticized solid state electrode using 2B graphite pencil rod with chitosan as coating material. The proposed method would then be investigated for the analysis of glutamate in food samples by flow injection analysis (FIA).

2. Experimental

2.1. Chemicals and reagents

High-molecular weight poly (vinyl chloride) (PVC), aspartic acid, ascorbic acid, citric acid and sodium benzoate were obtained from Fluka Chemika (Switzerland). Analytical reagent grade sodium chloride, sodium acetate and tetrahydrofuran (THF) were obtained from Merck (Germany). Analytical reagent grade lysine sodium salt of L-glutamic acid and glycine were obtained from BDH chemicals (England). All solutions were prepared using pure water (18.2 MΩ cm) from Milli–Q Plus, Millipore Corp. (USA). The pH adjustments were made with 0.1 M hydrochloric acid and 0.1 M sodium hydroxide. A stock solution of glutamate was freshly prepared by dissolving appropriate amount of analytical grade sodium salt of L-glutamatic acid from BDH chemicals (England) in pure water. The chitosan PM 100 (granular, 100 mesh) was obtained from Chito Chem. (Malaysia) and was used without purification.

2.2. Apparatus

The FIA system employed is shown in Fig. 1. Solution was propelled by a multi-channel peristaltic pump from Gilson Miniplus 3 (France) through PTFE tubing (0.8 mm i.d.). Samples were injected into a low-pressure four way rotary injection valve model 5020, Rheodyne (USA). The injection volume and carrier flow-rate used were 50 μl and 2.5 ml min^{-1}, respectively. A flow-through cell of the wall jet design Model FIP-3 with an in-built Ag/AgCl reference electrode was supplied by Chemflow Devices (Australia). All the flow-cell bodies (3.5 × 2.5 cm) were made of Perspex®. Recording of potentiometric peaks was performed on a single chart recorder; Kipp and Zonen model BD 111 (Holland). The potentiometric measurements were obtained using a pH/mV meter; model 720A from Orion Research Inc. (USA). The high performance liquid chromatograph (HPLC) from Varian ProStar (USA) was fitted with column adsorbosphere OPA HR from Altech (USA).

2.3. Pretreatment the of graphite rod electrode

The composite graphite rod, i.e. pencil lead was obtained by completely removing the wooden outer part of the 2B pencil Stabilo Micro 288 (Germany), using a sharp knife. The composite graphite was transferred into an appropriate thimble and placed into soxhlet extractor for 24 h continuous reflux in methanol. Subsequently, the graphite was washed in 0.1 M HCl and thoroughly rinsed with water prior to 10 min ultrasonic cleansing. The composite graphite was then dried in room temperature (25 ± 3 °C) and kept in desiccators until used.

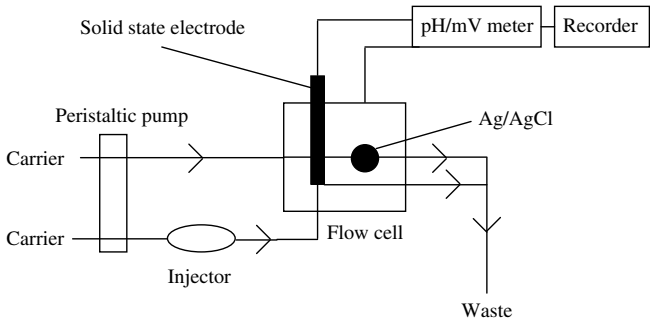

Fig. 1. The layout of the FIA system.

2.4. Electrode preparation

The general procedure to prepare the heterogeneous PVC membrane was adopted by mixing thoroughly 50 mg powdered PVC and 50 mg chitosan in 2 ml of THF (Isa & Ab Ghani, 2007). The solvent was evaporated slowly until an oily sludge concentrated mixture was obtained. The graphite rod was, then, immersed in the sludge mixture. The obtained solid state electrode was then kept at room temperature, following the 1 h complete evaporation of THF. Finally, the manufactured solid state electrode was inserted into the flow cell and then conditioned by continuously running in 10^{-6} M glutamate at pH 4 for 1 h.

2.5. Measurement of glutamate in food samples

All food samples tested were obtained from local supermarkets. The powdered solid samples had to be treated to decompose any available fat. This was done by pouring 1 g of the solid into 100 ml water and then heated at ~70 °C for 10 min, before cooling it of at room temperature. The solution was later filtered through Whatman No. 1 (Khampa et al., 2004). The supernatant was, then, diluted with pure water. (All the samples had to be diluted at least a thousand times prior to injection into the FIA system. The optimum dilution of the coloured samples was obtained when the solution became visually colourless. Thereafter, the samples were further diluted for the response to fall within the linear range of the standard glutamate solution). The results obtained were compared with those of the standard method for glutamate determination.

3. Result and discussion

3.1. Flow injection-system optimization and pH effect

It has been reported (Yang, Hibbert, & Alexander, 1998) that the sensitivity, selectivity and peak heights of graphite solid state electrode in an FIA system depends significantly on the membrane composition, especially the plasticizers. There are also reports (Heng & Hall, 2000 and Isa & Ab Ghani, 2007) highlighting on the better response of the membrane without plasticizer. Hence, this study has adopted the non-plasticizer approach a solid state electrode fabrication whereby chitosan is the substrate coating material. In another report (Zahir & Ab Ghani, 1997) from this laboratory the graphite pencil paste is found to perform better than the graphite pencil rod. In this experiment, however, the result is the opposite i.e. the composite graphite rod performs better than the composite graphite paste. The anomaly is probably due to the structural formation of the electrode which could have an effect on fluid dynamic within the FIA flow cell. Obviously, the composite rod is more rigid and compact than the composite paste.

The effects of sampling volume and carrier flow-rate on the magnitude of the signal (as peak height) are studied by injecting 1.0×10^{-3} M glutamate into the FIA system. The utilization of bigger sampling volumes and lower flow rates has enhanced response. The optimum values obtained for flow rate and sampling volume are 1.5 mL/min and 75 μL, respectively. The pH profile of the electrode in the pH range of 2–6 in a 1.0×10^{-3} M glutamate is shown in Fig. 2. Hence, pH 4.0 is the optimum pH. At pH 7 and higher, the peaks are broader and lower. The lowering in response is due to competition between co-ions glutamate and hydroxyl to the site in chitosan.

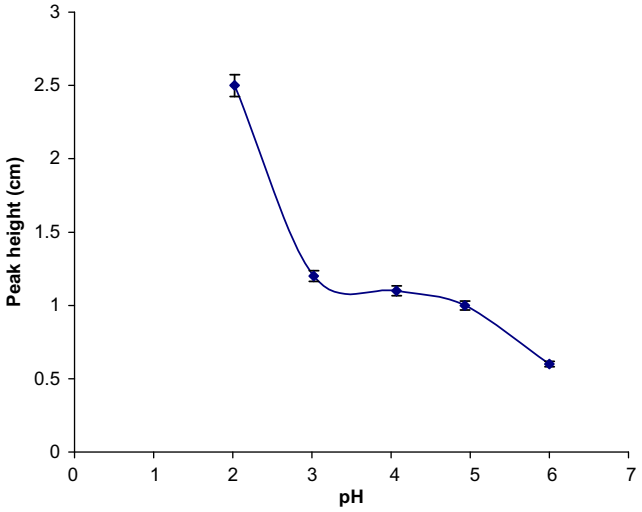

Fig. 2. The effect of pH on the peak height ($n = 3$).

3.2. Linear range and sensitivity of the assay

In most FIA experiments the composition of the carrier solution could influence the base line stability of the electrode used. Fig. 3 shows that the selected carrier solution, 6×10^{-6} M HCl (pH 5.2) has produced a stable base line for standard glutamate solutions with concentration range of 10^{-5} M to 10^{-2} M. A limit of detection (LOD) at 0.008 mM glutamate and linear range of 0.01–1 mM glutamate are obtainable.

The advantages of using the proposed solid state electrode for glutamate analysis is that it is relatively more user-friendly, robust and at a much lower cost (Khampa et al., 2004; Lau & Mok, 1995; Oliveira et al., 2001). Table 1 shows results of various electrodes in the literature. It shows that the result obtained by the proposed electrode is comparable to other methods.

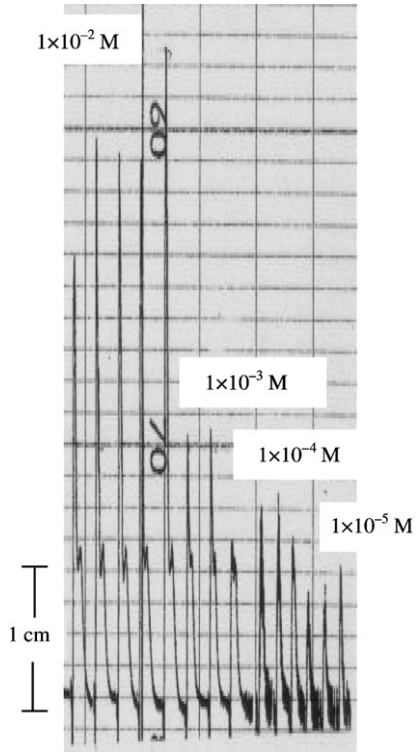

Fig. 3. The FIA peak heights of standard glutamate solutions.

Table 1
Glutamate analyses by the proposed method and others

	Linear range	Limit of detection
Proposed	0.01–1 mM	0.008 mM
Lau and Mok (1995)	0–2.76 mM	0.007% (w/w)
Khampa et al. (2004)	0.0002–0.01 mM	0.00014 mM
Oliveira et al. (2001)	2.5–75 mM	–

3.3. Specificity and interference

It is reported (Mizutani, Kimizuka, Ruddle, & Ishige, 1992) that in the analysis of glutamate in fish and soya sauces and in the presence of high concentration of other amino acids the recovery values become impaired. This study has, however, investigated on the recovery values of glutamate in the presence of not only other amino acids but also food additives and preservatives. This is performed by mixing 50 mmol of glutamate with 50 mmol of interferent prior to injection into the FIA system. Table 2 shows that no significant interference is observed from the interferents tested, indicating a high degree of specificity of the proposed method. Thus, the electrode is then applied in the analysis of glutamate in real samples.

3.4. Measurement of glutamate in foodstuffs

The food samples used are in powdered form. The labels on the package have only stated that they contain MSG but without mentioning the exact quantity. Table 3 shows the results obtained by the proposed and the standard method. It appears that the proposed method correlates well, $R^2 = 0.9981$, with the standard method (HPLC). In general, the amount of glutamate available in the foods samples is acceptable as has been stipulated by the Foods Act and Food Regulation (Ministry of Health, 1996).

Table 2
The recovery values of glutamate in the presence of interferences

Interferences	Recovery (%)[a]
Sodium chloride	90.1 ± 1.2
Sodium Benzoate	88.9 ± 0.7
Sodium acetate	92.2 ± 0.7
Ascorbic acid	88.8 ± 1.2
Citric acid	94.6 ± 0.7
Aspartic acid	93.7 ± 0.7
Lysine	89.9 ± 1.0
Glycine	99.2 ± 1.2

[a] All the values were the mean of triplicate measurements ($n = 3$).

Table 3
Analysis of glutamate in food samples by the proposed and the standard method (HPLC)

Powder Sample	Glutamate (%)[a]	
	Proposed method	HPLC
Seasoning (Tumix from Aji-no-moto)	21.3 ± 0.4	23.3 ± 0.4
Seasoning (Seri-Aji from Aji-no-moto)	13.6 ± 0.3	14.7 ± 0.2
Seasoning (Maggi cube from Nestle)	4.8 ± 0.2	5.2 ± 0.2
Chicken soup (Knorr from Unilever)	5.8 ± 0.2	5.4 ± 0.2
Chinese soup (Maggi from Nestle)	7.8 ± 0.3	7.7 ± 0.2
Mushroom soup (Vono from Aji-no-moto)	2.8 ± 0.2	2.5 ± 0.2

[a] All the values were the mean of triplicate measurements ($n = 3$).

4. Conclusions

A solid state electrode based on chitosan has been developed for the analysis of glutamate. The electrode is applied for the quantification of glutamate in food samples. However, the solution of the sample has to be fat-free to allow better mobility of dissolved glutamate ion to the electrode. The results indicate that the proposed method is comparable to the standard method (HPLC). In general, the proposed method is, moderately, better than the standard method in terms of reliability, simplicity and cost.

Acknowledgements

The authors would like to thank Universiti Sains Malaysia for Grants 131/0250/0580 and 304/PKIMIA/637063. One of us (Isa, I.M.) is indebted to Universiti Pendidikan Sultan Idris (UPSI) for granting study leave.

References

Bogdanov, M. B., Tjurmina, O. A., & Wurtman, R. J. (1996). Consumption of a high dietary dose of monosodium glutamate fails to affect extracellular glutamate levels in the hypothalamic arcuate nucleus of adult rats. *Brain Research, 736*, 76–81.

Cattrall, R. W., Drew, D. M., & Hamilton, I. C. (1975). Some alkylphosphoric acid esters for use in coated-wire calcium-selective electrodes: Part I. Response characteristics. *Analytica Chimica Acta, 76*, 269–277.

Chang, K. S., Hsu, W. L., Chen, H. Y., Chang, C. K., & Chen, C. Y. (2003). Determination of glutamate pyruvate transaminase activity in clinical specimens using a biosensor composed of immobilized L-glutamate oxidase in a photo-crosslinkable polymer membrane on a palladium-deposited screen-printed carbon electrode. *Analytica Chimica Acta, 481*, 199–208.

Chapman, J., & Zhou, M. (1999). Micro-plated based fluorometric methods for the enzymatic determination of L-glutamate: Application in measuring L-glutamate in food samples. *Analytica Chimica Acta, 402*, 47–52.

Guanghau, L., Xin, Y., Xiaogang, W., & Tong, Z. (2001). Determination of the total iron by chitosan-modified glassy carbon electrode. *Microchemical Journal, 69*, 81–87.

Halmos, G., Lendvai, B., Gaborjan, A., Baranyi, M., Szabo, L. Z., & Vitez, C. (2002). Simultaneous measurement of glutamate and dopamine release from isolated guinea pig cochlea. *Neurochemistry International, 40*, 243–248.

Hanko, V. P., & Rohrer, J. S. (2004). Determination of amino acids in cell culture and fermentation broth media using anion-exchange chromatography with integrated pulsed amperometric detection. *Analytical Biochemistry, 324*, 29–38.

Heng, L. Y., & Hall, E. A. H. (2000). Taking plasticiser out of methacrylic–acrylic membranes for K^+-selective electrodes. *Electroanalysis, 12*, 187–193.

Inoue, K., Baba, Y., Yashizuka, K., Noguchi, H., & Yoshizaki, M. (1988). Selectivity series in the adsorption of metal ions on a resin prepared by crosslinking copper (II)-complexed chitosan. *Chemistry Letters, 17*, 1281–1284.

Isa, I. M., & Ab Ghani, S. (2007). Development of prototype heterogeneous chitosan membrane using different plasticizer for glutamate sensing. *Talanta, 71*, 452–455.

Khampa, W., Meevootisom, V., & Wiyakrutta, S. (2004). Spectrophotometric enzymatic cycling method using L-glutamate dehydrogenase and D-phenylglycine aminotranferase for determination of L-glutamate in food. *Analytica Chimica Acta, 520*, 133–139.

Lau, O. W., & Mok, C. S. (1995). Indirect conductometric detection of amino acids after liquid chromatographic separation. Part II. Determination of monosodium glutamate in foods. *Analytica Chimica Acta, 302*, 45–52.

Ling, D., Wu, G., Wang, C., Wang, F., & Song, G. (2000). The preparation and characterization of an immobilized L-glutamic decarboxylase and its application for determination of L-glutamic acid. *Enzyme and Microbial Technology, 27*, 516–521.

Lu, M. J., Chiu, T. C., Chang, P. L., Ho, H. T., & Chang, H. T. (2005). Determination of glycine, glutamine, glutamate, and γ-aminobutyric acid in cerebrospinal fluids by capillary electrophoresis with light-emitting diode-induced fluorescence detection. *Analytica Chimica Acta, 538*, 143–150.

Ministry of Health, Malaysia (1996). *Food acts 281 and food regulations*. Kuala Lumpur: International Law Book Services.

Mizutani, T., Kimizuka, A., Ruddle, K., & Ishige, N. (1992). Chemical components of fermented fish products. *Journal of Food Composition and Analysis, 5*, 152–159.

Niwa, O., Horiuchi, T., Kurita, R., Tabei, H., & Torimitsu, K. (1998). Microfabricated on-line sensor for continuous monitoring of *l*-glutamate. *Analytical Sciences, 14*, 947–953.

No, H. K. (1987). Application of crawfish chitosan as a coagulant in recovery of organic compound from seafood processing wastes. Dissertation abstract (Int.) AAI 8811429.

Oliveira, M. I. P., Pimentael, M. C., Montenegro, M. C. B. S. M., Araujo, A. N., Pimentel, M. F., & da Silva, V. L. (2001). L-glutamate determination in food samples by flow-injection analysis. *Analytica Chimica Acta, 448*, 207–213.

Qu, J., Chen, W., Luo, G., Wang, Y., Xiao, S., & Ling, Z. (2002). Rapid determination of underivatized pyroglutamic acid, glutamic acid, glutamine and other relevant amino acids in fermentation media by LC-MS-MS. *Analyst, 127*, 66–69.

Riccardo, A., Muzzarelli, R. A., & Rochetti, R. (1974). Enhanced capacity of chitosan for transition-metal ions in sulphate-sulphuric acid solutions. *Talanta, 21*, 1137–1143.

Ryan, M. R., Lowry, J. P., & O'Neill, R. D. (1997). Biosensor for neurotransmitter L-glutamic acid designed for efficient use of L-glutamate oxidase and effective rejection of interference. *Analyst, 122*, 1419–1424.

Senstad, C., & Almas, K. A. (1986). *Chitin in Nature and Technology* (3rd ed.). In Muzzarelli R. A., Jeuniaux C. G., & Graham W. (Eds.), Proceedings of International Conference on Chitin and Chitosan (pp. 568–570). Plenum: New York, USA.

Tcherkas, Y. V., & Denisenko, A. D. (2001). Simultaneous determination of several amino acids, including homocysteine, cysteine and glutamic acid, in human plasma by isocratic reversed-phase high-performance liquid chromatography with fluorometric detection. *Journal of Chromatography A, 913*, 309–313.

Van Hemelrijck, A., Sarre, S., Smolders, I., & Michotte, Y. (2005). Determination of amino acids associated with cerebral ischaemia in rat brain microdialysates using narrowbore liquid chromatography and fluorescence detection. *Journal of Neuroscience Methods, 144*, 63–71.

Yang, X., Hibbert, D. B., & Alexander, P. W. (1998). Flow injection potentiometry by poly (vinyl chloride)-membrane electrodes with substituted azacrown ionophores for the determination of lead (II) and mercury (II) ions. *Analytica Chimica Acta, 372*, 387–398.

Zahir, M. H., & Ab Ghani, S. (1997). Fabrication of directly polymerized 4-vinylpyridine onto a pencil 2B graphite paste electrode for glucose monitoring. *Analytica Chimica Acta, 354*, 351–358.