

# A Stable Glutamate Biosensor Based on MnO<sub>2</sub> Bulk-modified Screen-printed Carbon Electrode and Nafion® Film-immobilized Glutamate Oxidase

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## ABSTRACT

An amperometric glutamate biosensor was developed using screen-printed carbon electrodes bulk-modified with MnO<sub>2</sub> (5%, m:m) onto which glutamate oxidase was immobilized via Nafion® film entrapment. The analytical performance of the biosensor was assessed in a flow injection mode and peak heights of the current response were used to evaluate results. Best responses were recorded at an applied potential of 440 mV (*vs* Ag/AgCl) a flow rate of 0.2 mL min<sup>-1</sup>, and a pH of 7.75 of the carrier (0.1 mol L<sup>-1</sup> phosphate buffer). The calibration curve exhibited linearity in the concentration range 10–160 mg L<sup>-1</sup>, with a detection limit (as 3σ value) of 1.7 mg L<sup>-1</sup>, and a relative standard deviation 3.3% (*c* = 20 mg L<sup>-1</sup>, *n* = 10). This biosensor was used for the determination of monosodium glutamate in food seasonings, and the values obtained were similar to those obtained with spectrophotometry. The biosensor exhibited extraordinary stability when left on the FI system at a flow rate of 0.1 mL min<sup>-1</sup> at room temperature retaining 50% of the original response towards glutamate even after 65 days. Stored in the working buffer for more than 60 days, the same biosensor showed extended linear range, 20–710 mg L<sup>-1</sup>. This 'aged' (stored) biosensor was used to determine monosodium glutamate in food seasonings and gave similar result to those obtained with a freshly prepared biosensor.

## KEYWORDS

Monosodium glutamate (MSG), amperometric glutamate biosensor, flow injection analysis, screen-printed electrodes, MnO<sub>2</sub>-modified electrodes.

## 1. Introduction

The amino acid L-glutamic acid or its salt L-glutamate is known to function as a substrate for protein synthesis and glutathione production, as a precursor of glutamine and N-acetylglutamate, as a neurotransmitter, as active sites of enzymes, as an inhibitor of glutaminase reaction, as an intermediate in the Citric Acid Cycle and as source of energy for some tissues in the body.<sup>1</sup> Owing to its ubiquitous nature, metabolic fate and vast function in the body it is implicated in the pathology and physiology of neurological and psychological diseases such as motor neuron, Huntington's, Parkinson's and Alzheimer's diseases.<sup>2</sup> This amino acid is also a product in transamination reactions catalysed by the enzymes glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) that are indicators in the diagnosis of hepatic diseases, myocardial infarction, and muscular dystrophy.<sup>3</sup> L-glutamic acid exists in most processed and raw foods in either the free form or bound to peptides and proteins.<sup>4</sup> Its sodium salt, monosodium glutamate (MSG), is added to several foodstuffs and seasonings to enhance the flavour. Idiosyncratic intolerance towards MSG has been observed in a sensitive sub-population and it is commonly known as Chinese Restaurant Syndrome.<sup>5</sup> Thus, the existence of a simple, accurate and reliable method of glutamate determination is vital in the biomedical sciences and food industries.

The determination of glutamate has been performed by spectrophotometry,<sup>6,7</sup> fluorometry,<sup>8,9</sup> chromatography,<sup>10–13</sup> electrophoresis,<sup>14–18</sup> potentiometry,<sup>19–22</sup> and amperometry.<sup>23–32</sup> Recently, more attention has been given to its amperometric

determination through enzymatic oxidation because the other methods have drawbacks with undesirable sensitivity, technical demand, high cost or are inconvenient to perform in a high-throughput format.

The amperometric determination of glutamate via oxidation or reduction current of hydrogen peroxide (one of the oxidation products of glutamate catalysed by glutamate oxidase) has been known for decades.<sup>32</sup> However, due to the high working potential required to oxidize hydrogen peroxide (e.g. +600 mV *vs* Ag/AgCl), interference from other electro-oxidizable species pose a problem, demanding the incorporation of mediators that shuttle electrons between the intermediately reduced enzyme and the electrode.<sup>33,34</sup>

In an attempt to use manganese dioxide (MnO<sub>2</sub>) as a mediator in amperometric determination of hydrogen peroxide, extensive research has been done, mostly in our laboratory, and MnO<sub>2</sub> film bulk-modified carbon paste and screen-printed amperometric sensors for H<sub>2</sub>O<sub>2</sub>,<sup>35–38</sup> uric acid,<sup>39</sup> and ascorbic acid<sup>40</sup> have been developed. Moreover, glucose biosensors have been designed based on carbon paste electrodes (CPEs) and screen-printed carbon electrodes (SPCEs) bulk-modified with MnO<sub>2</sub> and glucose oxidase (GOD).<sup>41–43</sup> At an operational potential of 400–500 mV *vs* Ag/AgCl, heterogeneous carbon electrodes bulk-modified with 3.8–5% MnO<sub>2</sub> responded to H<sub>2</sub>O<sub>2</sub> (either directly present in the sample or as a product of the enzymatic oxidation). The tetravalent manganese is reduced to lower oxidation states by H<sub>2</sub>O<sub>2</sub> and is re-oxidized again electrochemically.

Attempts to produce glutamate biosensors in similar fashion (both CPEs and SPCEs) have been unsuccessful.<sup>41</sup> Moreover,

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bulk-modification of the paste or the carbon ink with the enzyme is not cost-effective in the case of the most expensive oxidases such as glutamate oxidase since relatively large quantities of these enzymes are required. In an independent study to optimize and characterize immobilization of oxidases (e.g. glucose oxidase) in Nafion<sup>®</sup> films, it has been found that employing a neutralized solution of the polymer gives a higher current response and better inter-electrode reproducibility than diluted or as-received Nafion<sup>®</sup> showing good compatibility with MnO<sub>2</sub> bulk-modified electrode system.<sup>44</sup>

This paper reports the development and characterization of an extraordinarily stable amperometric glutamate biosensor based on screen-printed MnO<sub>2</sub> bulk-modified carbon electrode onto which glutamate oxidase was immobilized via Nafion<sup>®</sup> film.

## 2. Experimental

### 2.1. Materials

L-Glutamate oxidase, GIOD (EC 1.4.3.11 from *Streptomyces* sp., 12.1 U mg<sup>-1</sup> solid) was from Sigma Chemicals Co., (St. Louis, MO, USA). L-glutamate, monosodium salt monohydrate, was from Fluka (Fluka Chemie, Buchs, Switzerland). Nafion<sup>®</sup>, perfluorinated ion-exchange resin, 5% (w/w) solution in lower aliphatic alcohols and water was from Aldrich (Aldrich-Chemie GmbH & Co KG, Steinheim, Germany). All other chemicals used were analytical reagent grade.

### 2.2. Reagents and Solutions

The water used was double-distilled in a quartz still and deionized with an ion exchange system (Nanopure, Barnstead). Phosphate buffer (0.1 mol L<sup>-1</sup>) was prepared by mixing aqueous solutions (0.1 mol L<sup>-1</sup>) of sodium di-hydrogen phosphate (Fluka) and di-sodium hydrogen phosphate (Fluka) to produce solutions of the required pH. A stock solution of glutamate (5000 mg L<sup>-1</sup>) was prepared by dissolving 0.5 g L-glutamic acid monosodium salt monohydrate (Fluka) in 100 mL of the corresponding working buffer solution and stored at 4°C when not in use. Solutions of lower concentrations were prepared immediately before use.

### 2.3. Electrode Preparation

Carbon ink (4.75 g, Gwent C50905D1, Pontypool, UK) and MnO<sub>2</sub> (0.25 g, Merck) were thoroughly mixed manually for 30 min and then sonicated at room temperature for 30 min. The resulting mixture was immediately used for electrode fabrication. The working electrodes were prepared by screen-printing the MnO<sub>2</sub>-modified ink onto an inert laser pre-etched ceramic support (113 × 166 × 0.635 mm, No. CLS 641000396R, Coors Ceramics GmbH, Chattanooga, TN, USA). Thick layers of the modified carbon ink were formed by brushing the ink through an etched stencil (thickness 100 μm, electrode printing area 105 mm<sup>2</sup>) with the aid of the squeegee of a screen-printing device (SP-200, MPM, Franklin, MA, USA) on to the ceramic substrates. The resulting plates were dried at 60°C for 1 h.

### 2.4. Enzyme Immobilization

Glutamate oxidase was immobilized according to a recent report.<sup>44</sup> Nafion<sup>®</sup> (5% solution) was neutralized to pH ~7 by a drop of ammonia solution (Fischer Scientific). A vial of GIOD containing 0.43 mg solid was dissolved in 20 μL 0.1 mol L<sup>-1</sup> phosphate buffer (pH 7.5) and mixed with equal amount of neutralized Nafion<sup>®</sup> solution. Ten μL of the resulting mixture was directly applied onto the active area of the screen-printed electrode (MnO<sub>2</sub> bulk-modified) surface (~0.40 cm<sup>2</sup> area), air

dried, another 10 μL aliquot applied, air dried and introduced in the FIA system.

### 2.5. Flow Injection System

The flow injection system consisted of a high performance liquid chromatographic (HPLC) pump (510 Waters, Milford MA, USA) in conjunction with a system controller (Waters 600E), a sample injection valve (5020 Rheodyne, Cotati, CA, USA), and a thin-layer electrochemical detector (LC-4C, BAS, West Lafayette, Indiana, USA) with a flow through cell (CC-5, BAS) in combination with the electrochemical workstation BAS 100B. The working electrode was fixed using Teflon gaskets directly to the back plate of the thin-layer cell with a Teflon support as a holder. Silver conductive paint (Electrolube Ltd, Wargrave, Berkshire, UK) was applied on one end of the SPCE, to which a crocodile clamp was attached for electrical contact. The reference electrode was Ag/AgCl (3 mol L<sup>-1</sup> KCl, Model RE-6, BAS) and the counter-electrode the stainless steel back plate of the cell. The software BAS 100 W ver. 2 was employed for data processing. The responses were evaluated using peak height.

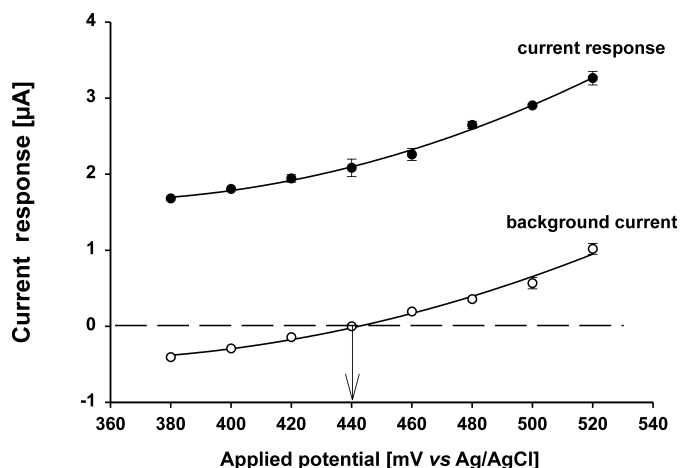
### 2.6. Determination of Monosodium Glutamate in Food Seasonings

'Aromat', 'Kräuterlinge' (both from C.H. Knorr GmbH, Wels, Austria) food seasonings and 'Frühlingsuppe' vegetable soup (Dr. Lange & Co. GmbH, Duesseldorf, Germany) were purchased from a local supermarket. The first two were homogeneous powders but from the soup only the powdery part, which was believed to contain MSG, was taken, leaving aside the other particulate ingredients. Twenty mg of the powder were dissolved in 10 mL phosphate buffer (0.1 mol L<sup>-1</sup>, pH 7.75), filtered through a syringe filter (Cameo 25NS, 0.22 μm pore size, Osmonics Inc., USA), diluted 10-fold (1 + 9) with the same buffer and analysed by injecting 100 μL of the diluted sample to the FI system. For recovery studies, 50 mg L<sup>-1</sup> of standard glutamate were spiked to the diluted sample before injection. The results were evaluated by the peak height of the current responses and values read from the corresponding calibration curve.

The spectrophotometric determination was made by using the colour reagent from Wako Chemicals (Neuss, Germany) that contained sodium salt of N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine (0.64 mmol L<sup>-1</sup>), peroxidase (2000 U L<sup>-1</sup>), 4-aminoantipyrine (0.6 mmol L<sup>-1</sup>), ascorbate oxidase (4000 U L<sup>-1</sup>) and uricase (40 U L<sup>-1</sup>). To 20 mL of the colour reagent 0.2 mg of GIOD was added ('glutamate color reagent'). To 600 μL of the glutamate colour reagent, 10 μL of the 10-fold (1 + 9) diluted sample extract was added, incubated for 10 min at 37°C and the absorbance of the colour developed was read at 546 nm (against a reagent blank) using a UV/VIS spectrophotometer (U-1500, HITACHI, Hitachi Instruments Inc., USA). Standards were run with the same procedure and the amount of MSG in the sample was evaluated from the calibration curve.

## 3. Results and Discussion

It has previously been demonstrated that the optimum concentration of MnO<sub>2</sub> is 3.8–5% (m: m) and the optimum potential 400–500 mV vs Ag/AgCl.<sup>35–44</sup> Thus, a 5% modifier was used in this work. Unlike the unsuccessful attempts by Turkusic to develop a glutamate biosensor by bulk-modifying MnO<sub>2</sub>-modified CPEs and SPCEs with GIOD, an electrode with the enzyme immobilized in a Nafion<sup>®</sup> film responded to injections of 250 μL standard glutamate solutions at a working potential of 480 mV vs Ag/AgCl and flow rate of 0.2 mL min<sup>-1</sup> of the carrier



**Figure 1** Dependence of the current response of glutamate on the applied potential. Working conditions: flow rate  $0.2 \text{ mL min}^{-1}$ , concentration of standard glutamate solution  $53 \text{ mg L}^{-1}$ , injection volume  $250 \mu\text{L}$ , carrier phosphate buffer ( $0.1 \text{ mol L}^{-1}$ , pH 7.5). Measurements were done in triplicate.

( $0.1 \text{ mol L}^{-1}$  phosphate buffer pH 7.5). In an independent study to optimize and characterize use of Nafion<sup>®</sup> film for immobilization of oxidases onto the active surface of SPCEs,<sup>44</sup> it was demonstrated that employing neutralized Nafion<sup>®</sup> gives higher current response and good inter-electrode reproducibility than diluted or as-received Nafion<sup>®</sup> and therefore, the same protocol was used to immobilize GIOD.

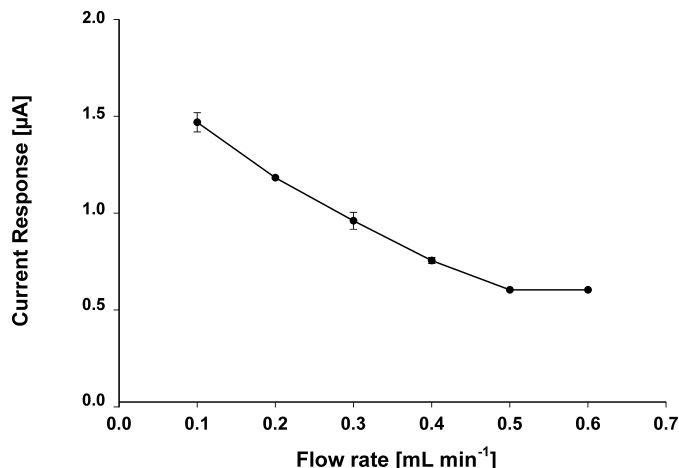
### 3.1. Optimization of Operational Parameters

#### 3.1.1. Applied Potential

Schachl *et al.* demonstrated that  $\text{MnO}_2$  bulk-modified electrodes show highest response in the potential range 400–500 mV *vs* Ag/AgCl.<sup>38,43</sup> Figure 1 shows the dependence of current response and background current on the applied potential. At 440 mV *vs* Ag/AgCl the background current is nearly zero and the current response higher than at lower potentials. This is in close agreement with the  $\text{MnO}_2$  bulk-modified CPEs and SPCEs that exhibited highest response in the potential range 400–500 mV *vs* Ag/AgCl,<sup>38,43</sup> as well as the glucose biosensor produced by Nafion<sup>®</sup> film-immobilized GOD.<sup>44</sup> The slight difference of the operating potential as compared to glucose oxidase (i.e. 400 mV *vs* Ag/AgCl) is probably due to slightly different physical properties of the membrane caused by the modification with glutamate oxidase. At potentials lower than 440 mV, decreasing response was observed and the background current was reductive, which may lead to gradual leaching out of the modifier due to formation of soluble Mn(II) species. At potentials higher than 440 mV, even though increasing response was obtained, the background current was also increasing that may have affected reproducibility of measurements. Thus, a working potential of 440 mV was chosen for subsequent measurements.

#### 3.1.2. Flow Rate

Like any FI measurement, the amperometric current response was affected by change in the flow rate (Fig. 2). The peak height exhibited an inverse relationship with flow rate. i.e. the higher the flow rate the lower the current response. At higher flow rates the residence time of the analyte in close proximity to the biological recognition element (the enzyme) was very small and dispersion was higher hence lower peak height. With a constant

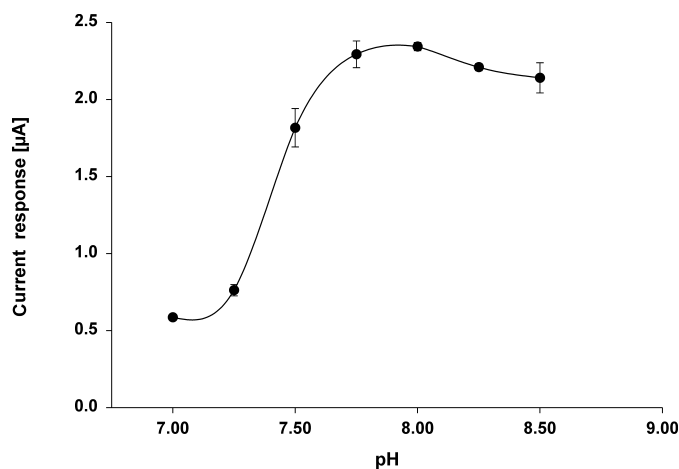


**Figure 2** Dependence of the current response on the flow rate. Working conditions: applied potential of 440 mV *vs* Ag/AgCl, concentration of standard glutamate solution  $50 \text{ mg L}^{-1}$ , injection volume  $250 \mu\text{L}$ , carrier phosphate buffer ( $0.1 \text{ mol L}^{-1}$ , pH 7.5); ( $n = 3$ ).

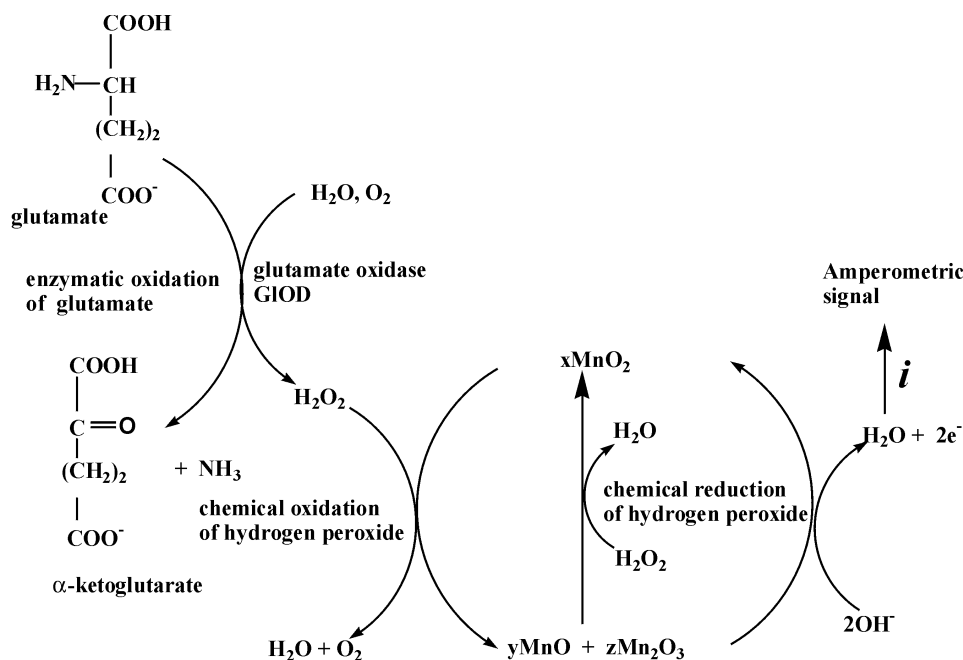
concentration of analyte in the carrier one would expect an increase of the signal with the flow rate (proportional to the cube root of the flow rate for a thin-layer cell). As the sample is injected, only a transient signal is obtained, which is determined by the dispersion (increasing with higher flow rates) and the kinetics of the enzymatic reaction. One may expect better sensitivity at lower flow rates than tested in this work ( $0.1\text{--}0.6 \text{ mL min}^{-1}$ ) but doing so would significantly prolong the analysis time of one sample, which is about 3 min. Among the flow rates tested the highest signal was obtained at  $0.1 \text{ mL min}^{-1}$  but due to the long relaxation time required and hence the slow sample throughput,  $0.2 \text{ mL min}^{-1}$  was chosen as working parameter.

#### 3.1.3. pH of the Carrier

Both the enzymatic and the electrochemical reactions are dependent on pH. Highest signal was observed at pH 8.0 (Fig. 3). At higher pH values ( $\sim 9.0$ ) chemical reduction of  $\text{H}_2\text{O}_2$  as a competing reaction (Fig. 4) to the electrochemical reoxidation of  $\text{MnO}_2$  may prevail.<sup>38,43</sup> To avoid such a risk, a pH of 7.75 was taken as working pH.



**Figure 3** Dependence of the current response on the pH of carrier ( $0.1 \text{ mol L}^{-1}$  phosphate buffer). Working conditions: applied potential of 440 mV *vs* Ag/AgCl, concentration of standard glutamate solution  $50 \text{ mg L}^{-1}$ , flow rate  $0.2 \text{ mL min}^{-1}$ , injection volume  $250 \mu\text{L}$ . Measurements were done in triplicate.



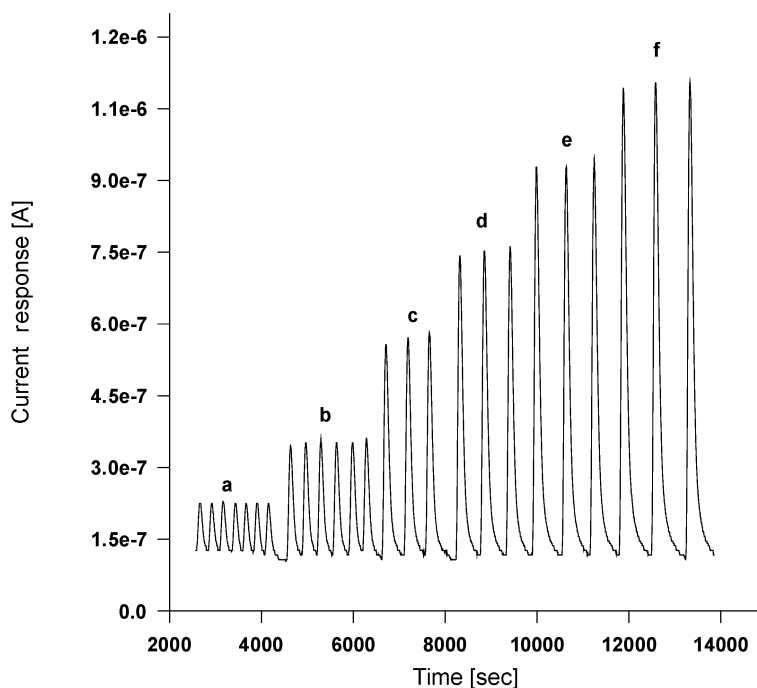
**Figure 4** Reaction mechanism of the oxidation of glutamate catalysed by GLOD immobilized in a Nafion<sup>®</sup> film on MnO<sub>2</sub> bulk-modified screen-printed carbon electrodes.

### 3.2. Figures of Merit

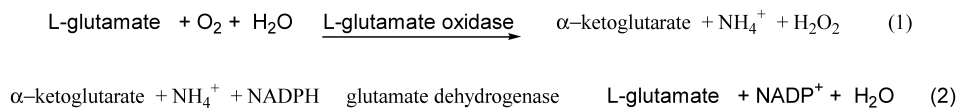
Figure 5 shows typical responses of the biosensor to injections of 250  $\mu$ L of different concentrations of standard glutamate solutions. Employing the operational parameters discussed above, linear relation ( $i [\mu\text{A}] = 0.023c[\text{mg L}^{-1}] + 0.04$ ,  $r^2 = 0.994$ ) between concentration and current response was observed in the range 10–160  $\text{mg L}^{-1}$  (Fig. 6A). At concentrations above 160  $\text{mg L}^{-1}$  there was deviation from linearity that might be due to limited access of molecular oxygen essential for the enzymatic reaction.<sup>45</sup> The detection limit (as  $3\sigma$  values) from five injections of 250  $\mu$ L standard glutamate solution (10  $\text{mg mL}^{-1}$ ) was found to be 1.7  $\text{mg mL}^{-1}$ . A relative standard deviation of 3.3% was recorded for 10 injections of 20  $\text{mg mL}^{-1}$  glutamate. In comparison

to previous electrochemical reports,<sup>23,25,27–29,31,32</sup> the linear range in this work was far better though the detection limit was a bit higher. The higher detection limit could be attributed to the diffusion barrier created by the Nafion-enzyme layer. As the thickness of layers increases the linear range extends but the detection limit becomes higher as reported elsewhere.<sup>46,47–49</sup> It can be further improved by employing an enzymatic substrate recycling method<sup>28,32</sup> according to the reactions in Scheme 1.

According to Yao *et al.*<sup>28</sup> and Villarta *et al.*<sup>32</sup>, co-immobilizing GLOD and glutamate dehydrogenase [EC 1.4.1.4], may cause cycling of L-glutamate,  $\alpha$ -ketoglutarate, and NH<sub>4</sub><sup>+</sup> between the enzymatic reactions, in the presence of NADPH, resulting in an increase in the rate of H<sub>2</sub>O<sub>2</sub> production which may result in sig-



**Figure 5** Typical response of a glutamate biosensor developed for concentrations of (a) 10, (b) 20, (c) 40, (d) 60, (e) 80 and (f) 100  $\text{mg L}^{-1}$  glutamate at an applied potential of 440 mV vs Ag/AgCl, flow rate of 0.2  $\text{mL min}^{-1}$ , carrier phosphate buffer (0.1  $\text{mol L}^{-1}$ , pH 7.75).



Scheme 1  
Reaction scheme

● freshly prepared biosensor: linear range 10-160 mg L<sup>-1</sup>, r<sup>2</sup> = 0.994  
▲ biosensor stored in the working buffer: linear range 10-710 mg L<sup>-1</sup>, r<sup>2</sup> = 0.997  
■ biosensor left online for 65 days: linear range 10-150 mg L<sup>-1</sup>, r<sup>2</sup> = 0.994

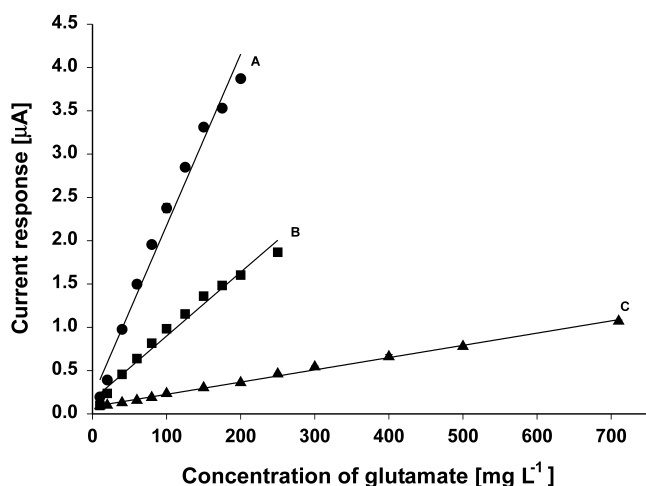


Figure 6 Calibration curve for different concentrations of glutamate using: (A) a freshly prepared biosensor; (B) a biosensor stored for more than two months in the working buffer solution; and (C) a biosensor kept on-line in the FI system for 65 days. Working conditions: applied potential of 440 mV vs Ag/AgCl, flow rate 0.2 mL min<sup>-1</sup>, injection volume 100 µL, carrier phosphate buffer (0.1 mol L<sup>-1</sup>, pH 7.75). Measurements were done in triplicate.

nificant improvement in the detection limit. However, it needs to be tested with the sensor developed in this work.

### 3.3. Storage

After having been used continuously for five days in a FI mode, the biosensor was stored in the working phosphate buffer for more than two months. After this period of storage, it was reintroduced in the FI system and gave an exploitable response, though lower than freshly prepared sensors. It exhibited an extended linear range ( $i[\mu\text{A}] = 0.0014c[\text{mg L}^{-1}] + 0.084$ ,  $r^2 = 0.997$ ) for concentrations from 10 to 710 mg L<sup>-1</sup> (Figure 6C). The detection limit, calculated as 3σ values from eight injections of 100 µL (10 mg L<sup>-1</sup>) standard glutamate solution, was 5.1 mg L<sup>-1</sup>. The relative standard deviation for five injections of 100 µL standard glutamate solution (20 mg L<sup>-1</sup>) was 1.7%.

Another freshly prepared biosensor, after having been used for measurement of glutamate for two weeks, was left in the flow injection system at a flow rate of 0.1 mL min<sup>-1</sup> at room temperature to study its long-term stability. Its response was checked regularly by injecting 100 µL of standard glutamate solution (500 mg L<sup>-1</sup>). Assuming that the reference electrode worked satisfactorily (i.e. ignoring fluctuations with depleting chloride concentration), after 65 days it retained almost 50% of the original response (Fig. 7). The calibration curve of this biosensor after 65 days is shown in Fig. 6B. The extraordinary stability of

Table 1 Monosodium glutamate content in food seasonings and a soup sample as determined by the biosensor and spectrophotometry.

Sample	Amount of monosodium glutamate (mass %) determined by:	
	Amperometry	Spectrophotometry
Knorr Aromat	17.36 ± 0.05	20.24 ± 0.21
Knorr Kraeuterlinge	19.65 ± 0.41	21.16 ± 0.25
Soup powder	22.19 ± 0.34	23.84 ± 0.53

this biosensor might be due to the MnO<sub>2</sub> incorporated in the electrode system. Vikartovska-Welwardova *et al.* reported such stabilizing effect of MnO<sub>2</sub> on D-amino acid oxidase from *Trigonopsis variabilis*.<sup>50</sup> However, it requires further investigation to ascertain the effect of MnO<sub>2</sub> on GIOD.

### 3.4. Determination of Monosodium Glutamate in Knorr Aromat Seasoning

The glutamate biosensor was used for the determination of monosodium glutamate (MSG) in food seasonings and soup powders. The results were compared with an enzymatic spectrophotometric method (Table 1). For the latter, the same enzyme, GIOD, was used for the oxidation of glutamate but the product, hydrogen peroxide, was detected by its colour reaction with N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine in the presence of 4-aminoantipyrine. In all samples slightly higher values were obtained with the spectrophotometric method that may be due to small amounts of ascorbic acid in the sample that might have been oxidized by ascorbate oxidase in the colour reagent (see Experimental). But otherwise the values fell within acceptable limits.

The old biosensor stored in phosphate buffer was also used to determine MSG in Knorr Aromat food seasoning. The corre-

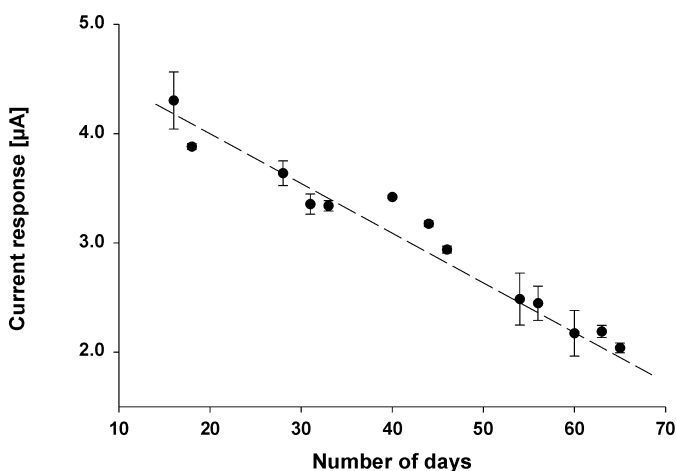


Figure 7 Long-term stability of glutamate biosensor left on-line on FI system as monitored by injection of 100 µL of 500 mg L<sup>-1</sup> standard glutamate solution. Error bars were calculated for triplicate measurements and working conditions were the same as in Fig. 6.

sponding concentration from the calibration curve gave  $16.66 \pm 0.02$  (mass %) MSG in the sample that is still in good agreement with the value obtained by using the freshly prepared biosensor. A recovery test was performed by spiking  $50 \text{ mg L}^{-1}$  standard glutamate into the diluted sample solution. The recovery was found to be  $102 \pm 2\%$ .

#### 4. Conclusions

To sum up, the new glutamate biosensor developed by immobilizing GIOD in a Nafion<sup>®</sup> film on MnO<sub>2</sub> bulk-modified SPCE exhibited a wider linear range than in previous works<sup>23,25,27–29,31,32</sup> and good storage stability. Its practicality was demonstrated by determining the amount of MSG in food seasoning. The advantages of screen-printing technology (mass production of inexpensive, reproducible and sensitive electrochemical sensors) in combination with the ease of immobilization technique employed, retaining of exploitable activity after more than two months in storage, and further improvement in linear range after storage renders this biosensor a promising candidate in food industry and in biomedical sciences.

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#### References

- 1 V.R. Young and A.M. Ajami, *J. Nutr.*, 2000, **130**, 892S–900S.
- 2 B.S. Meldrum, *J. Nutr.*, 2000, **130**, 1007S–1015S.
- 3 D.W. Moss and A.R. Henderson, in *Tietz Textbook of Clinical Chemistry*, (C.A. Burtis and E.R. Ashwood, Eds.), W.B. Saunders, Philadelphia, PA, USA 1999, pp. 652–653.
- 4 S. Garattini, *J. Nutr.* 2000, **130**, 901S–909S.
- 5 J.R. Lupien and R. Walker, *J. Nutr.*, 2000, **130**, 1049S–1052S.
- 6 U. Mankasingh, D. Narinesingh and T.T. Ngo, *Anal. Lett.*, 2000, **33**, 2407–2423.
- 7 E. Valero and F. Garcia-Carmona, *Anal. Biochem.*, 1998, **259**, 265–271.
- 8 M. Zhou and J. Chapman, *Anal. Chim. Acta*, 1999, **402**, 47–52.
- 9 G.G. Guilbault and B. Rietz, *Anal. Chim. Acta*, 1975, **77**, 191–198.
- 10 N. Ioannou, A. Georgopoulos, C. Liapi and G. Palaiologos, *Anal. Biochem.*, 1998, **264**, 82–86.
- 11 M.B. Bogdanov, O.A. Tjurmina and R.J. Wurtman, *Brain Res.*, 1996, **736**, 76–81.
- 12 L.W. Anderson, D.W. Zaharevitz and J.M. Strong, *Anal. Biochem.*, 1987, **163**, 358–368.
- 13 G. Fortier, D. Tenaschuk and S.L. MacKenzie, *J. Chromatogr.*, 1986, **361**, 253–261.
- 14 H. Wie and S.F.Y. Li, *Anal. Chem.*, 1998, **70**, 5097–5102.
- 15 M.W. Lada, T.W. Vickroy and R.T. Kennedy, *Anal. Chem.*, 1997, **69**, 4560–4565.
- 16 M.W. Lada and R.T. Kennedy, *Anal. Chem.*, 1996, **68**, 2790–2797.
- 17 S.Y. Zhou, H. Zuo, J.F. Stobaugh, C.E. Lunte and S.M. Lunte, *Anal. Chem.*, 1995, **67**, 594–599.
- 18 T.M. O'Shea, P.L. Weber, B.P. Bammel, C.E. Lunte and S.M. Lunte, *J. Chromatogr.*, 1992, **608**, 181–188.
- 19 *Official Methods of Analysis* (1998) 16th edn., 4th revision, 1998, AOAC International, Gaithersburg, MD, method 970.37.
- 20 T. Gündüz, N. Gündüz and E. Kilic, *Analyst*, 1988, **113**, 715–719.
- 21 W. Meng-Liang and W. Chang-Yi, *Anal. Chim. Acta*, 1987, **198**, 325–328.
- 22 E. Athanasiou-Malaki and M.A. Koupparis, *Analyst*, 1987, **112**, 757–761.
- 23 Y. Yigzaw, L. Gorton and T. Solomon, *Curr. Separations*, 2002, **19**, 119–125.
- 24 T. Yao, Y. Nanjyo and H. Nishino, *Anal. Sci.*, 2001, **17**, 703–708.
- 25 A.K. Karyakin, E.E. Karyakina and L. Gorton, *Anal. Chem.*, 2000, **72**, 1720–1723.
- 26 O. Niwa, R. Kurita, T. Horiuchi and K. Torimitsu, *Electroanalysis*, 1999, **11**, 356–361.
- 27 F. Mizutani, Y. Sato, Y. Hirata and S. Yabuki, *Biosens. Bioelectron.*, 1998, **13**, 809–815.
- 28 T. Yao, S. Suzuki, T. Nakahara and H. Nishino, *Talanta*, 1998, **45**, 917–923.
- 29 S. Ghobadi, E. Csöregi, G. Marko-Varga and L. Gorton, *Curr. Separations*, 1996, **14**, 94–102.
- 30 I. Moser, J. Jobst, E. Aschauer, P. Svasek, M. Varahram and G. Urban, *Biosens. Bioelectron.*, 1995, **10**, 527–532.
- 31 N.F. Almeida and A.K. Mulchandani, *Anal. Chim. Acta*, 1993, **282**, 353–361.
- 32 R.L. Villarta, D.D. Cunningham and G.G. Guilbault, *Talanta*, 1991, **38**, 49–55.
- 33 K. Habermüller, M. Mosbach and W. Schuhmann, *Fresenius J. Anal. Chem.*, 2000, **366**, 560–568.
- 34 A. Chaubery and B.D. Malhorta, *Biosens. Bioelectron.*, 2002, **17**, 441–456.
- 35 K. Schachl, H. Alemu, K. Kalcher, H. Moderegger, I. Svancara and K. Vytras, *Fresenius J. Anal. Chem.*, 1998, **362**, 194–200.
- 36 K. Schachl, H. Alemu, K. Kalcher, J. Jezkova, I. Svancara and K. Vytras, *Sci. Pap. Univ. Pardubice, Ser. A*, 1997, **3**, 41–55.
- 37 K. Schachl, H. Alemu, K. Kalcher, J. Jezkova, I. Svancara and K. Vytras, *Anal. Lett.*, 1997, **30**, 2655–2673.
- 38 K. Schachl, H. Alemu, K. Kalcher, J. Jezkova, I. Svancara and K. Vytras, *Analyst*, 1997, **122**, 985–989.
- 39 A. Chopra, *Development of an Amperometric Sensor for the Determination of Uric Acid*, M.Sc. thesis, University of Sarajevo, Sarajevo, Bosnia and Herzegovina, 2001.
- 40 E. Turkušić, V. Milićević, H. Tahmišćija, M. Vehabović, S. Bašić and V. Amžić, *Fresenius J. Anal. Chem.*, 2000, **368**, 466–470.
- 41 E. Turkušić, *Development of Some New Amperometric Biosensors*, Ph.D. dissertation, University of Sarajevo, Sarajevo, Bosnia and Herzegovina, 2001.
- 42 E. Turkušić, K. Kalcher, K. Schachl, A. Komersova, M. Bartos, H. Moderegger, I. Svancara and K. Vytras, *Anal. Lett.* 2001, **34**, 2633–2647.
- 43 K. Schachl, *Development of Electrochemical Sensors Based on Carbon Electrodes Modified with Manganese Dioxide*, Ph.D. dissertation, Karl-Franzens University of Graz, Graz, Austria, 1998.
- 44 N.W. Beyene, *Development of Sensors and Biosensors for Some Biologically Relevant Compounds*, Ph.D. dissertation, Karl-Franzens University of Graz, Graz, Austria, 2003.
- 45 I.L. Mattos, L.V. Lukachova, L. Gorton, T. Laurell and A.A. Karyakin, *Talanta*, 2001, **54**, 963–974.
- 46 A. Maines, D. Ashworth and P. Vadgama, *Anal. Chim. Acta*, 1996, **333**, 223–231.
- 47 D.J. Harrison, R.F.B. Turner and H.P. Baltes, *Anal. Chem.*, 1988, **60**, 2002–2007.
- 48 W.H. Mullen, F.H. Keedy, S.J. Churchouse and P.M. Vadgama, *Anal. Chim. Acta*, 1986, **183**, 59–66.
- 49 A. Maines, M.I. Prodromidis, S.M. Tzouwara-Karayanni, M.I. Karayannis, D. Ashworth and P. Vadgama, *Electroanalysis*, 2000, **12**, 1118–1123.
- 50 A. Vikartovska-Welwardova, E. Michalkova, P. Gemeiner and L. Welward, *Folia Microbiol.*, 1999, **44**, 380–384.