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L-Glutamate biosensor for estimation of the taste of tomato specimens

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Abstract An amperometric biosensor has been developed for measurement of Umami, or the taste based on the amount of L-glutamate, in tomato foods. The biosensor is based on an enzyme-mediator system in which L-glutamate oxidase is used for biochemical oxidation of L-glutamate and a tetrafulvalene-tetracyanoquinodimethane (TTF-TCNQ) paste, prepared from the mixture of TTF-TCNQ salt, graphite powder, and silicone oil, serves as the mediator. The limit of detection, calculated by use of a four-parameter logistic model, was 0.05 mmol L⁻¹, and the limit of quantification was 0.15 mmol L⁻¹. The correlation coefficient (R^2) was 0.990 and the relative standard deviation was no more than 1% ($n=5$). The response time (τ_{95}) was 20–50 s, depending on concentration. The repeatability of the sensor was better than 5% ($n=10$). The sensor developed was stable for more than ten days.

Keywords Amperometric biosensors · Umami · L-Glutamate determination · L-Glutamate oxidase · TTF-TCNQ

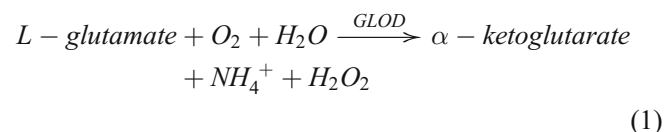
Introduction

L-Glutamic acid and its monosodium salt (monosodium glutamate, MSG) give an enjoyable taste—normally referred to as Umami—to food. Some kinds of vegetable, for example corn and ripe tomatoes contain quite large amounts of MSG (130–140 mg per 100 g vegetable). MSG

is also added to food products for seasoning. Because the method currently used for determination of MSG is very old and quite complicated [1, 2] a new and faster method is required for quality control of tomato food products, e.g. by determination of Umami. Artificial electronic tongues could be used for that purpose [3].

The first electrochemical glutamate biosensor based on glutamate dehydrogenase and coenzyme NAD⁺ was introduced by Malinauskas and Kulys in 1978. Oxidoreductase mixed with immobilized NAD⁺ cofactor was held between a suitable platinum electrode and a semi-permeable membrane and the coenzyme was readily regenerated either directly by electrochemical oxidation or by using phenazine methosulfate as intermediate [4]. Although L-glutamate oxidase (GLOx) is usually used for development of amperometric L-glutamate biosensors [5–13], glutamate dehydrogenase is still widely used for the same purpose [8, 14–18]. Because noble metals are usually used as the electrode material, GLOx is attached to them by use of different polymer membranes, e.g. a cellulose acetate dialysis membrane [6, 7], Nafion [10], electrochemically deposited polyphenyldiamine [19, 20], poly(ethylene glycol) diglycidyl ether [21], a photo-crosslinkable polymer (PVA-SbQ) [22], polycarbamoylsulfonate [11], a redox polymer Os-gel containing horseradish peroxidase (HRP) [12, 13, 23], Os-polyvinylpyridine [24], or [Os(4,4'-dimethylbipyridine)₂Cl] [25]. GLOx has also been combined with other enzymes to increase its stability and activity, e.g. with a redox polymer containing HRP [12, 13, 23], glutamate dehydrogenase [8], or ascorbic acid oxidase to prevent interference [10].

Glutamate oxidase oxidises L-glutamate to α -ketoglutarate and releases hydrogen peroxide and ammonium ions:



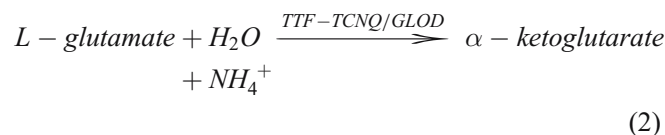
The product of the enzymatic reaction, H₂O₂, can be detected electrochemically by reduction or by oxidation [6]. MnO₂ has also been used as the mediator for indirect

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glutamate determination, i.e. by detecting hydrogen peroxide [26]. Direct determination of the substrate, however, results in better stability of the enzyme and a more precise response of the sensor. For this purpose different mediators–electron-transfer complexes, e.g. Os complexes [12, 13, 23–25] or tetrafulvalene-tetracyanoquinodimethane complex (TTF-TCNQ) [26, 27]—were introduced for direct substrate oxidation:



Mediators transfer electrons from the active site of the enzyme directly to the electrode and, therefore, reduce the oxidation potential of the substrate and regenerate the co-enzyme (FAD if GLOx is used) [29, 30].

In this paper, the preparation of a biosensor for determination of L-glutamate in tomato food samples is reported¹. The biosensor is based on glutamate oxidase cross-linked to a TTF-TCNQ mediator paste as an electron-transferring material for direct analyte determination. The enzyme is regenerated by the mediator.

Experimental

Chemicals and solutions

Tetrathiafulvalene (TTF), tetracyanoquinodimethane (TCNQ), graphite powder, high-temperature silicone oil AP 1000, bovine serum albumin (BSA), glycerol, glutaraldehyde, imidazole, LiCl, NaCl, KCl, HCl (conc.), LiOH, NaOH, MnCl₂, acetonitrile, L-glutamic acid, ascorbic acid, acetic acid, citric acid, malic acid, D-glucose, D-fructose, and amino acids (L-alanine, L-aspartic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine) were of puriss or analytical grade and purchased from Fluka (Buchs, Switzerland). L-Glutamate oxidase (GLOx) from *Streptomyces* sp. (lyophilised powder) was obtained from Seikagaku America (Falmouth, USA).

Doubly-distilled water was used for the preparation of all solutions. Imidazole buffer pH 7.0 (0.1 mol L⁻¹) was prepared by dissolving imidazole in water with addition of either LiCl (0.1 mol L⁻¹) or a mixture of NaCl (0.05 mol L⁻¹) and KCl (0.05 mol L⁻¹). The pH was adjusted by addition of dilute HCl solution (1:1 volume ratio). Standard L-glutamate solution (1 mol L⁻¹) was prepared by dissolving L-glutamic acid either in LiOH (1 mol L⁻¹) or NaOH (1 mol L⁻¹) solution. Both solutions were stored at +4 °C when not in use. 5% GLOx solution was prepared by dissolving L-glutamate oxidase and BSA (1:1 mass ratio) in 100 μL

imidazole buffer with addition of 5 μL glycerol. The GLOx solution was stored at -18 °C when not in use.

TTF-TCNQ (1:1 molar ratio of the salt) was prepared according to the procedure described by Jaeger and Bard [31] and was used without additional recrystallization.

Different certified tomato food samples from Unilever [32] were investigated to evaluate the L-glutamate biosensor developed.

Biosensor preparation

TTF-TCNQ paste was prepared by thoroughly mixing of TTF-TCNQ crystals, graphite powder, and silicone oil (3:2:3, mass ratio). The paste was manually pressed into a cavity of inner diameter 1.5 mm and depth 2.0 mm, in Teflon, on a top of a standard glassy carbon rotating disc electrode (RDE, Metrohm, Herisau, Switzerland) or automatically printed on a screen-printed electrode (SPE), where the diameter of the working electrode was 0.8 mm (Fig. 1). To cross-link the enzyme 0.5 μL GLOx solution was dropped with a micropipette on to the surface of the electrode. Aqueous MnCl₂ solution (1 mmol L⁻¹, 0.5 μL) was then dropped on the top of the enzyme layer and dried in air; another 0.5 μL of the GLOx solution was dropped on the top and the electrode was placed in glutaraldehyde vapour for 20 min at room temperature. The membrane formed was then dried in air for a few minutes and the electrode was then rinsed with doubly-distilled water and stored in buffer solution at +4 °C when not in use.

Instruments and procedure

The TTF-TCNQ paste with cross-linked GLOx on an RDE or a screen printed electrode (SPE) was used as the working electrode. An Ag/AgCl electrode (Metrohm, Herisau, Switzerland) filled with saturated KCl solution served as reference electrode and a Pt bar 7 cm long and 5 mm in diameter served as the counter electrode. Ag/AgCl paste and carbon ink, printed on the SPE (Fig. 1b), were also used as quasi-reference and counter electrode, respectively. The electrode system was controlled by a potentiostat PGSTAT20 driven by GPES3 software (Autolab, Utrecht, The Netherlands).

Measurements were performed at an operating potential of +0.05 V, either with a rotation rate of 500 rpm or with stirring of the solution instead of electrode rotation (with the SPE, only stirring of the solution was used) in 0.1 mol L⁻¹ imidazole buffer either with addition of 0.1 mol L⁻¹ LiCl (for general studies) or 0.05 mol L⁻¹ NaCl and 0.05 mol L⁻¹ KCl (for sample analysis) at pH 7.0 and 25±0.1 °C.

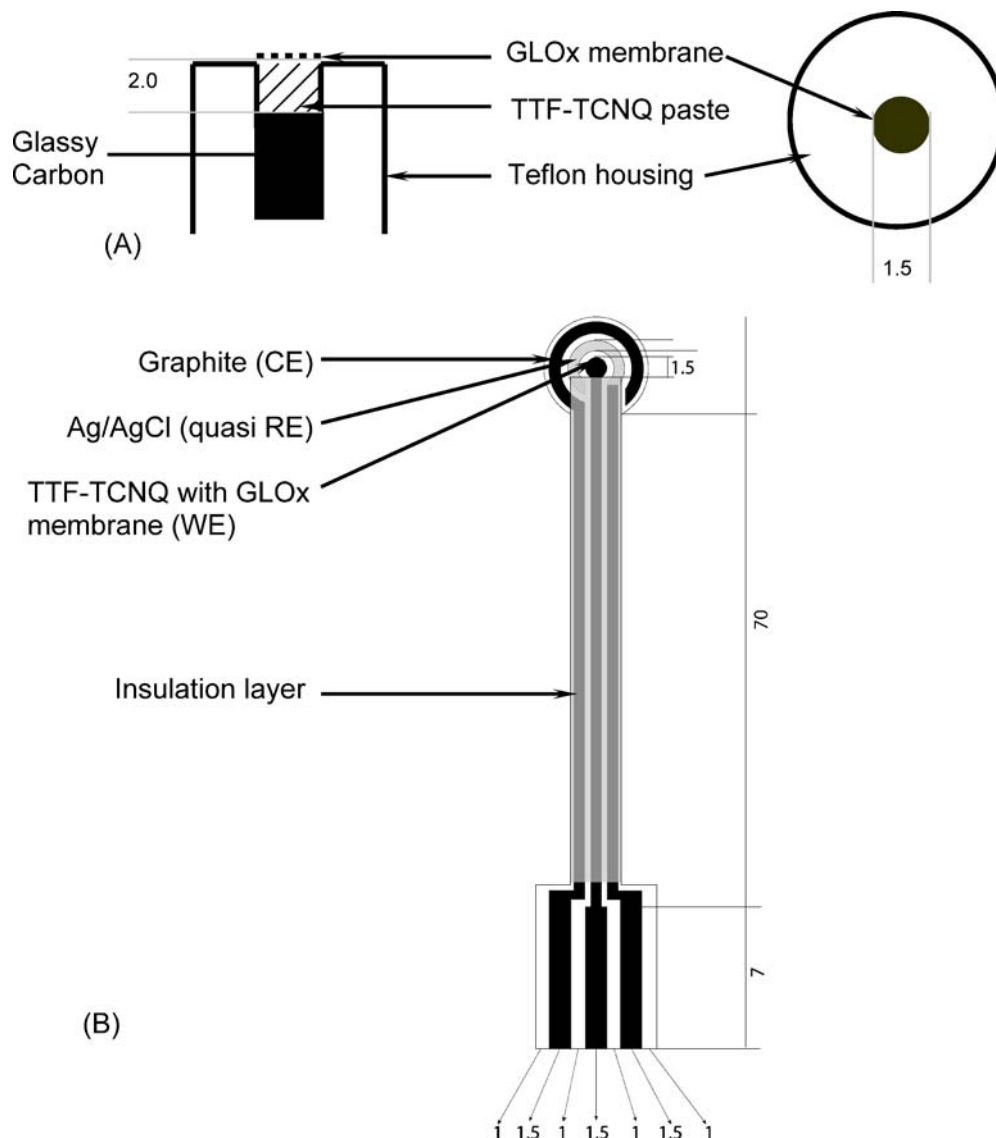
Results and discussion

Optimisation of the operating conditions

The response of an amperometric mediator-paste biosensor is a function of a number of conditions, for example the

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Fig. 1 Schematic diagrams of different types of L-glutamate sensor: (a) bulk TTF-TCNQ electrode; (b) screen-printed electrode (SPE)



composition of the mediating paste, the method used for immobilization of the enzyme, the operating potential, the supporting electrolyte, pH, ionic strength, etc. TTF-TCNQ was used as the mediator for electron transfer because it is a conductive, non-toxic organic salt capable of regenerating the active site of the enzyme [33]. Because TTF-TCNQ is expensive and sophisticated to use as an electrode material, the mediator was mixed with graphite and binder, for example high-temperature silicone oil, to form a conductive thick paste which was used as the base of the working electrode [22–24, 27, 28, 30–38]. The best composition ratio of TTF-TCNQ, graphite powder, and silicone oil was found to be 3:2:3 (mass ratio) [38]. GLOx was immobilised on the surface of the paste electrode by treatment with saturated glutaraldehyde vapour; no additional diffusion membrane was used. The best time for cross-linking with glutaraldehyde was found to be 20 min. The enzyme membrane was not formed if shorter cross-linking times were used and the activity of the enzyme

decreased exponentially when cross-linking was performed for longer than 20 min.

The electrochemical behaviour of the mediator-enzyme electrode is shown in Fig. 2. Electrochemical oxidation of TCNQ^- to TCNQ occurred from +0.05 to +0.40 V relative to Ag/AgCl in the positive-direction potential scan (Fig. 2, curve with 0 mmol L^{-1} glutamate), and TCNQ was reduced to TCNQ^- in the reverse potential scan. At more positive potentials in the positive scan direction oxidation of TTF to TTF^+ starts, and the opposite process occurs at more negative values than -0.10 V in the negative-direction potential scan [31, 39]. An oxidation wave at +0.25 V with continuous increase in current was observed on addition of glutamate to the buffer solution. At a certain concentration the current no longer changed on further addition of glutamate, because of over-saturation of the enzyme. The active part of GLOx is FAD, which is reduced to FADH_2 during reaction with the substrate. TCNQ oxidizes FADH_2 back to the initial form, FAD, and is itself reduced to

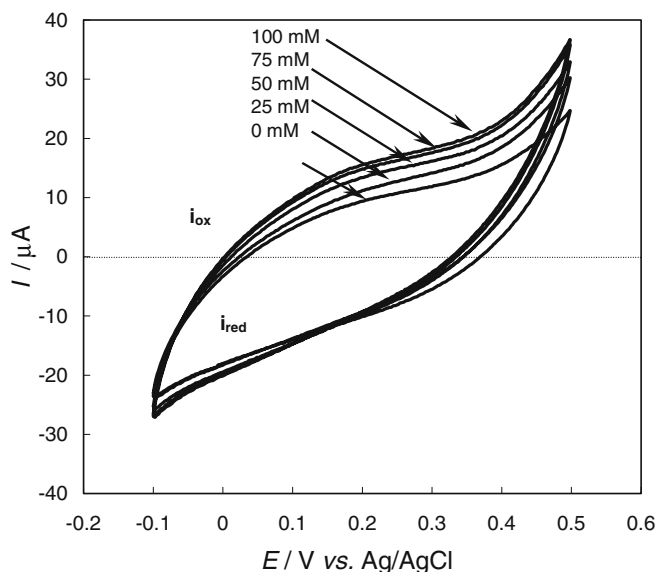


Fig. 2 Cyclic voltammograms obtained from different concentrations of L-glutamate by oxidation at a TTF-TCNQ paste-GLOx RDE electrode in a supporting electrolyte of 0.1 mol L⁻¹ imidazole buffer containing 0.1 mol L⁻¹ LiCl at pH 7.01. The rate of rotation was 500 rpm, the scanning rate 10 mV s⁻¹, and the temperature 25 °C

TCNQ⁻, thus regenerating the enzyme [29]. The constant current increase in the positive potential region shows, however, that the reaction rate between the electrode and the electroactive compound is the rate-limiting step. For this particular biosensor, this is probably because of competitive formation of hydrogen peroxide, because regeneration of the enzyme by the electrode material may be poor.

The response of the biosensor to glutamate also depended on the operating potential (Fig. 3). The background current slowly increased when the polarizing potential was increased from -0.05 to +0.15 V; the increase in current was greatest for potentials from +0.05 to +0.10 V, similar to the voltammetric behaviour of the same electrode (Fig. 2). The amperometric response to glutamate increased with increasing polarizing potential from -0.05 to +0.20 V (Fig. 3a), as is usual for amperometric behaviour when electron transfer occurs directly between the electrode and the interfaced enzyme [40]. The best operating potential for an amperometric sensor is when the response current to the substrate is sufficiently high and the background current is approximately 0 A; for this sensor the best operating potential was between +0.05 and +0.10 V, because the mediator was unstable after operation of the sensor for long periods at lower potentials (data not shown). The dependence of the logarithm of the actual response current on the potential indicated that the kinetics of substrate oxidation were not linear over the whole potential region studied, but had two linear ranges (Fig. 3b).

The supporting electrolyte also played an important role in the process of mediated enzymatic oxidation of L-glutamate. Phosphate buffer is usually used in biochemical reactions but was not appropriate in this work because of the formation of insoluble salts of TTF⁺ with the

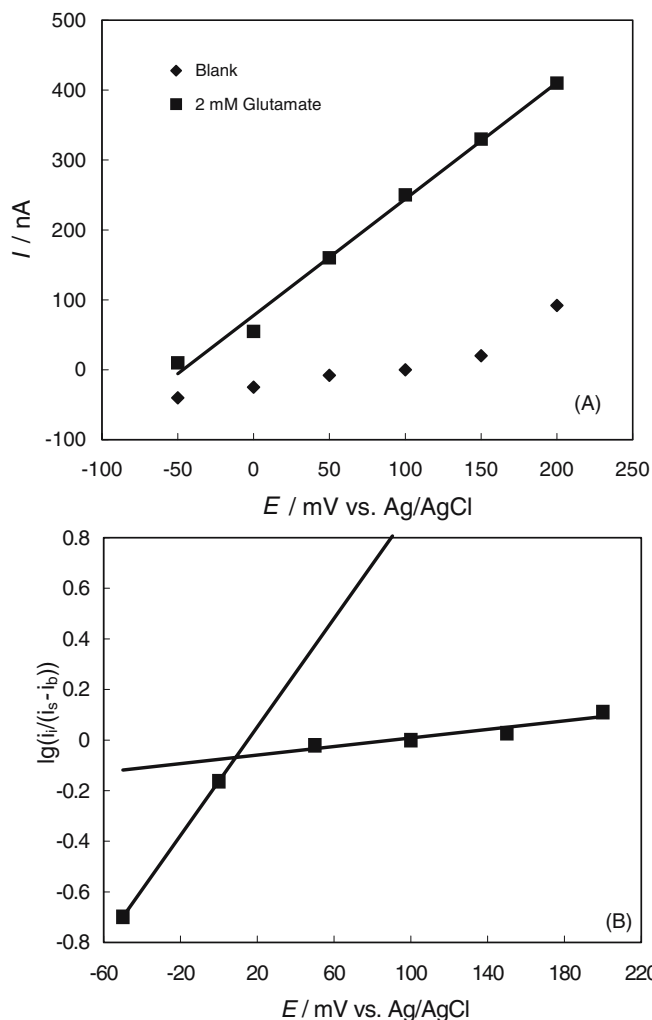


Fig. 3 (a) Amperometric response to 2 mmol L⁻¹ L-glutamate and (b) logarithmic actual response at different potentials. All other conditions were as for Fig. 1

phosphate anion [27]. Both imidazole and Tris-buffer were inert to the mediator and compatible with GLOx, but the L-glutamate oxidation signal was better while imidazole buffer was used, so that buffer was chosen. Because the conductivity of the organic buffer was not sufficient, KCl and NaCl were added. These specific salts were chosen as supporting electrolyte because of their presence in the tomato food samples [32]. The optimum pH for GLOx activity was between 7.0 and 7.5, as reported elsewhere [6–8, 11, 22]. Although experimental results indicated the strongest relative response was at pH 7.46 (physiological pH) (Fig. 4a), the background current was also highest at this pH, and pH 7.0 was chosen. This pH was also more suitable because of the nature of the samples—the pH of the tomato food products was 3.5–4.6, and to avoid unnecessarily high dilution of the samples during their neutralisation it was better to work at neutral pH. The electrochemical behaviour of the sensor at a solution pH of 7.0 was, moreover, similar to that at pH 7.46. Increasing the ionic strength, *H*, *I*, led to a continuous decrease of the signal (Fig. 4b) because of solvation of the ions of the supporting

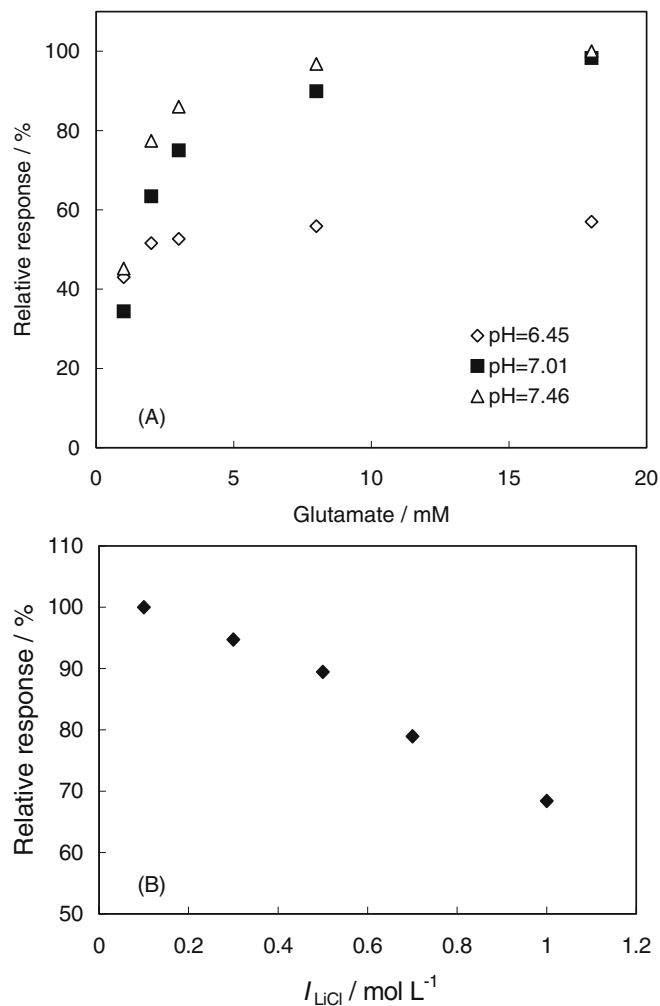


Fig. 4 Effect of buffer solution pH (a) and ionic strength (b) on L-glutamate amperometric oxidation at +0.10 mV relative to Ag/AgCl. All other conditions were as for Fig. 1

electrolyte, i.e. a salt effect [41]. The best response was obtained at $I=0.1 \text{ mol L}^{-1}$, and this was selected for further work. Because tomato food samples contain both NaCl and KCl, the ionic strength was adjusted by use of a 1:1 mixture of these salts.

Because of the complex nature of the samples, including different organic acids, sugars, and amino acids [32], it was important to check for interference from these compounds. The results obtained are presented in Table 1. Sugars and salts did not interfere with the glutamate response whereas citric acid and some amino acids resulted in slight interference. As expected, it was observed that even small concentrations of ascorbic acid resulted in a response several times larger than that from a few mmol L^{-1} of glutamate. It was already known that TTF-TCNQ catalyses the direct oxidation of ascorbic acid [35, 42]. It is a challenging task to eliminate this interference. Because deprotonated ascorbic acid (at the pH of the working solution) and the glutamate anion have the same charge and a similar ion size, a reduction of the interference by use of

Table 1 Effect of several compounds present in tomato food products on the glutamate response measured with an RDE consisting of TTF-TCNQ:C:silicone oil (3:2:3 mass ratio)-GLOx(20 min in glutaraldehyde vapour)

Compound	Concentration (mmol L ⁻¹)	Relative response (%)
Sugars		
Glucose	5	0
Fructose	5	0
Acids		
Acetic acid	5	0
Ascorbic acid	1	+444
	5	+1903
Citric acid	1	+4
	5	+20
Malic acid	5	-3
Mineral salts		
NaCl	5	0
KCl	5	0
Amino acids		
Alanine	5	0
Aspartic acid	5	0
Glutamine	5	+7
Histidine	1	+5
	5	+22
Glycine	5	+3
Isoleucine	5	0
Leucine	5	-3
Methionine	5	-7
Phenylalanine	5	+12
Proline	5	+0
Serine	5	-3
Threonine	5	0
Valine	5	-5
Tyrosine	1	-3
	5	-9

Samples were diluted with 0.1 mol L^{-1} imidazole buffer solution containing 0.05 mol L^{-1} NaCl, 0.05 mol L^{-1} KCl, and 2 mmol L^{-1} L-glutamate, pH 7.01. Measurements were performed at 25 °C, 500 rpm, and an operating potential of 100 mV relative to Ag/AgCl

Nafion or any positively charged or neutral polymeric membrane as a barrier was not possible. The amperometric response of the TTF-TCNQ paste electrode to ascorbic acid also depended on the polarization potential (Table 2) and the tendency was similar to that observed for glutamate—an increase with increasing potential (Fig. 3). In accordance with this tendency, the best potential for reducing the interference by ascorbic acid would be 0.0 V (the response to glutamate would be too low at -0.05 V), but when 0.0 V was used as the operating potential, the stability of the sensor was poor. Oxidation of ascorbic acid at a TTF-TCNQ electrode without a GLOx membrane was stable and directly proportional to the concentration of ascorbic acid. It was therefore possible to measure the glutamate

Table 2 Response to ascorbic acid at different operating potentials

Potential (mV)	-50	0	50	100	150	200
Response to 1 mmol L ⁻¹ ascorbic acid (nA)	130	290	420	560	600	650

All other conditions as for Table 1

response as the difference between the response for oxidation of ascorbic acid at a TTF-TCNQ electrode without GLOx and that for total oxidation of both analytes at a TTF-TCNQ/GLOx electrode:

$$I_{\text{Glut}} = I_{\text{total}} - I_{\text{AA}} \quad (3)$$

where I_{Glut} is the glutamate oxidation current, I_{total} the total oxidation current, and I_{AA} the oxidation current for ascorbic acid measured at the a TTF-TCNQ paste electrode without a GLOx membrane. Although the presence of the enzyme membrane did not reduce the response to ascorbic acid, the response time was slightly increased. It is, therefore, appropriate to use this difference method for determination of glutamate with elimination of the interference signal from ascorbic acid. A similar method has been used for determination of cysteine in the presence of ascorbic acid by use of an optical sensor [43].

The storage stability of the RDE biosensor (Fig. 1a) was up to 10 days during operation for 2–3 hours per day (Fig. 5); it is, therefore, one of the most stable L-glutamate biosensors. The biosensor was stored in imidazole buffer at +4 °C when not in use. In the initial phase, while the protein membrane was still not thoroughly wetted, the signal was slightly lower, but after use for one day a stable signal was observed for five days before the activity started

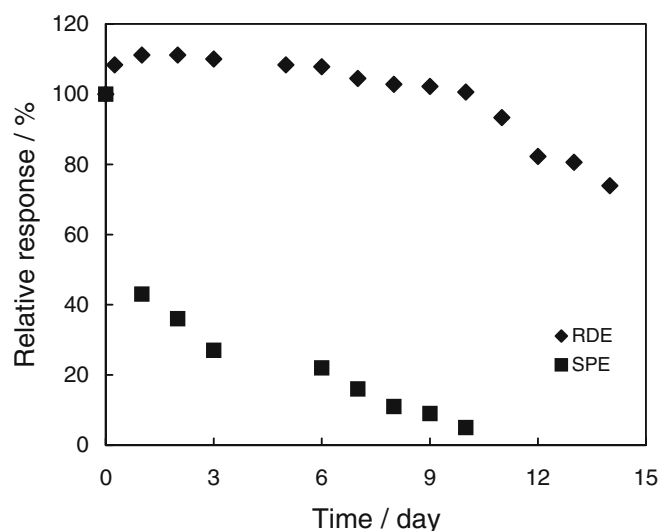


Fig. 5 Amperometric response to 1.5 mmol L⁻¹ L-glutamate of a rotating disc TTF-TCNQ paste electrode (*rhomboid symbols*) or of a screen-printed TTF-TCNQ paste electrode (*square symbols*) with cross-linked GLOx. The electrode was kept in buffer solution at +4 °C when not in use. Working conditions: 0.1 mol L⁻¹ imidazole buffer containing 0.05 mol L⁻¹ NaCl and 0.05 mol L⁻¹ KCl, pH 7.01. The operating potential was +0.05 V relative to Ag/AgCl, the rate of rotation was 500 rpm, and the temperature was 25 °C

to decrease slowly (Fig. 5, rhomboid symbols). Even after two weeks the sensor still retained approximately 60% of its initial activity. It was noticed that the activity depended on the thickness of the mediator layer—the sensor stability of the SPE (Fig. 1b) was poor and the activity decreased to 40% even on the first day of use (Fig. 5, square symbols). It seems that the stability depended on the surface roughness—the SPE surface was much smoother than that of the bulk paste electrode and there was poor mediator contact with the active centres of the enzyme [30]. For the SPE, it could also be related to the leaching of the mediator. Since the TTF-TCNQ layer was too thin, the decrease in the signal was faster than for the RDE. For the RDE, slight leaching of the mediator did not have too much effect because most of the mediator was in the bulk.

The sensor was calibrated by simple addition of standard solution to the buffer and supporting electrolyte. Because the calibration curve was sigmoidal in shape (Fig. 6), owing to the different mediation mechanisms (H₂O₂ formation and determination at low concentrations and direct electron transfer at higher concentrations), a four-parameter logistic model [44] was used to evaluate it. This very flexible calibration model can be expressed by the equation [44]:

$$y = d + \frac{a - d}{1 + (x/c)^b} \quad (4)$$

where x is the concentration, y is the current response, a is the lower asymptote, d is the higher asymptote, c is the IC₅₀, and b is the slope of the linearized curve (Fig. 6, inset). The parameters in this particular instance were

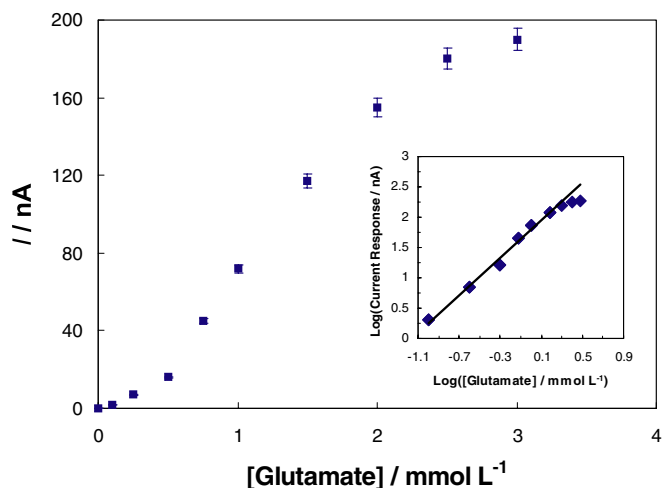


Fig. 6 Calibration plot for determination of L-glutamate with an RDE TTF-TCNQ paste electrode with cross-linked GLOx. All other conditions were as for Fig. 5

$a=0$ nA, $b=1.5$, $c(IC_{50})=1.3$ mmol L⁻¹, $d=200$ nA ($R^2=0.990$). The LOD calculated using this four-parameter logistic model [44] Eq. (4) was 0.05 mmol L⁻¹ and the LOQ was 0.15 mmol L⁻¹.

The response time for a 95% signal change (τ_{95}) was between 20 and 50 s, depending on the concentration of glutamate. The repeatability of the sensor, for measurements using the same electrode, was better than 5% ($n=10$). For different sensors the reproducibility was up to 15% ($n=5$).

Measurement of glutamate in tomato foods

Different kinds of certified tomato food products, e.g. pastes, ketchups, sauces, and juices were analysed for their glutamate content. The sensor was calibrated every day with “synthetic tomato food” calibrators prepared from compounds present in tomato food products at the concentrations declared by the manufacturers (Table 1), except for ascorbic acid [32]. Because the tomato food products were of low pH and GLOx is active only at neutral pH, the products were diluted with imidazole buffer to pH 7.0. The measured signal (difference between electrodes with and without GLOx, to eliminate interference from ascorbic acid) was compared with the calibration data. The results obtained and the apparent recovery data [45] are presented in Table 3. Occasionally, when the concentration of L-glutamate was greater than 40 mmol L⁻¹, the values obtained with the amperometric biosensor showed recovery to be greater than 100%, whereas the opposite tendency was observed at lower concentrations. The phenomenon could be related to the different viscosity of the samples—samples with a higher glutamate concentration were of higher viscosity. Although the tomato pastes were diluted, the suspensions were of higher viscosity than the synthetic calibration solutions. It would be possible to filter the diluted samples to remove solid matter, but the objective of this work was to pretreat the samples as little as possible. The sample solutions were diluted up to 50 times before measurement and solid particles did not block the electrode surface. Electrochemical sensors measure the concentration activity of the free analyte in a given amount of solvent, i.e. the molal concentration. For less viscous samples the amount of accessible solvent (water in this case) was higher [29]. The standard deviation mostly depended on sample characteristics such as viscosity, size of the solid particles, and homogeneity.

Conclusions

An amperometric sensor has been developed for determination of L-glutamate in low-pH, high-viscosity food samples, mainly tomato food products. It is based on an enzyme-mediator (GLOx and TTF-TCNQ) system. It was demonstrated that the sensor could be used to monitor glutamate in tomato products and probably in any other food. This method is simple and rapid, a large advantage

Table 3 Determination of L-glutamate in tomato food products with the amperometric biosensor

Number and type of certified sample	Dilution (times)	Sample pH	Declared amount of L-glutamate (mmol L ⁻¹)	L-Glutamate found with amperometric sensor (mmol L ⁻¹)	Apparent recovery (%)
1 (Juice)	20	4.6	15.9	12.7±1.3	79.9
2 (Paste)	50	4.5	55.4	58.2±1.2	105.1
3 (Juice)	10	4.6	11.3	7.3±0.3	64.6
4 (Paste)	40	4.4	41.1	32.0±3.5	77.8
5 (Paste)	50	4.4	51.0	65.4±0.6	128.2
6 (Paste)	40	4.3	41.8	39.8±2.9	95.2
7 (Paste)	40	4.4	42.5	38.0±1.1	89.4
8 (Paste)	40	4.3	41.5	37.0±2.1	89.1
9 (Paste)	40	4.4	51.0	48.5±4.7	95.1
10 (Paste)	40	4.5	47.1	31.6±2.6	67.1
11 (Paste)	40	4.3	45.7	49.5±0.8	108.3
12 (Ketchup)	10	3.7	8.2	7.7±0.3	93.9
13 (Ketchup)	10	3.8	8.2	9.9±2.1	120.7
14 (Ketchup)	20	3.9	15.6	14.4±0.1	92.3
15 (Sauce)	20	4.3	17.0	16.4±1.1	96.5
16 (Sauce)	20	4.3	13.6	12.0±1.6	88.2
17 (Sauce)	20	4.3	15.6	14.7±0.4	94.2
18 (Paste)	20	4.5	13.6	17.0±1.1	125.0
19 (Paste)	20	4.5	12.2	11.3±0.8	92.6
20 (Paste)	10	4.5	13.6	14.5±0.7	106.6
21 (Paste)	10	4.4	13.6	10.1±0.7	74.3
22 (Paste)	20	4.5	12.2	12.1±0.2	99.2
23 (Paste)	20	4.5	12.2	11.3±0.2	92.6
24 (Paste)	10	4.4	12.2	17.0±1.2	139.3
25 (Paste)	10	4.4	12.2	12.3±0.3	100.8
26 (Paste)	20	4.4	13.6	18.0±0.3	132.4
27 (Paste)	20	4.4	13.6	13.4±0.5	98.5
28 (Paste)	20	4.4	12.2	14.0±0.0	114.8
29 (Paste)	10	4.6	13.6	13.4±0.2	98.5
30 (Paste)	10	4.5	13.6	13.9±0.6	102.2

Samples were diluted with 0.1 mol L⁻¹ imidazole buffer containing 0.05 mol L⁻¹ NaCl and 0.05 mol L⁻¹ KCl at pH 7.01. The operating potential was 50 mV relative to Ag/AgCl, the rate of rotation was 500 rpm, and the temperature 25 °C

compared with the current reference method. It could also be developed in the food industry for quality control.

The tube sensor was stable for more than 10 days and is therefore one of the most stable glutamate sensors yet reported. A four-parameter logistic model was used to calculate the limits of detection and quantification of the sensor, because of the sigmoidal calibration curve obtained. The LOD was 0.05 mmol L⁻¹ and the LOQ was 0.15 mmol L⁻¹; these values are perfectly suitable for the samples analyzed in this study. R^2 was 0.990 and the relative standard deviation was no more than 1% (five measurements). The response time (τ_{95}) was between 20 and 50 s, depending on the glutamate concentration. Good apparent recovery for certified samples of tomato foods was obtained by use of the electrochemical biosensor.

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