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Accurate glutamate monitoring in foodstuffs by a sensitive and interference-free glutamate oxidase based disposable amperometric biosensor

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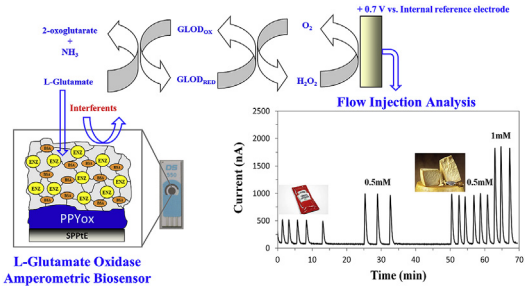
HIGHLIGHTS

- A new L-glutamate oxidase based biosensor was developed onto Pt disposable electrodes.
- The biosensor showed a very good sensitivity, linearity, selectivity, and response stability.
- The glutamate biosensor was very useful for routine analyses of complex food samples.

GRAPHICAL ABSTRACT



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ABSTRACT

L-Glutamate (L-Glu) is a well-known flavour enhancer that is present in several foodstuffs. Although L-Glu is generally recognized as safe, the use in foodstuffs remains controversial and then its fast and accurate monitoring represents an important issue. In this work a sensitive and interference-free disposable amperometric biosensor for glutamate monitoring in foodstuffs was developed. The biosensor was prepared by immobilizing glutamate oxidase through co-crosslinking with bovine serum albumin and glutaraldehyde onto a screen printed disposable platinum electrode modified with a permselective overoxidized polypyrrole film. The enzyme immobilization was optimized by using different experimental procedures. The optimized glutamate biosensor was integrated in a flow injection system and characterized in terms of linearity (0.005–1.0 mM, $r^2 = 0.992$), limits of detection (1.8 μM) and quantitation (5.4 μM), repeatability (RSD < 3%) and stability of response under operational conditions (up to 50 h, over 400 analysis). The biosensor showed also excellent anti-interference characteristics towards the main electroactive interferents present in food matrices, and this allowed the application to the accurate monitoring of glutamate in different foodstuffs.

1. Introduction

L-Glutamate (L-Glu) is a well-known flavour enhancer naturally present in several foodstuffs like cheese and seasonings. It is responsible for umami, which is one of the five basic tastes of the human sense of taste. Glutamic acid, as monosodium glutamate salt, is often used as an additive in savoury prepared and processed foods, such as spice mixes, frozen foods, meat or fish-based products, canned and dry soups and salad dressings. Although L-Glu is

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generally recognized as safe, the use in foodstuffs remains controversial. In fact, owing to an excessive intake of this flavour enhancer, a number of people have manifested the so-called Chinese restaurant syndrome, with allergy symptoms as headache and stomach pain [1]. Also, L-Glu is considered a strong neuroexcitatory amino acid associated with certain behavior patterns, such as aggressive behavior, visual-task learning [2], morphine-induced muscular rigidity [3], retrograde amnesia [4] and, recently, neurotoxic effects and excitotoxicity [5]. In the European Union [6], glutamates are classified as food additives with codes E620-625, and the relevant total concentration should not exceed 10 g kg^{-1}, while for some foodstuffs a maximum level of *quantum satis* has been set, where *quantum satis* has a stringent meaning [7]. Therefore, L-Glu monitoring is an important issue and then various methods have been developed, based mainly on chromatography [8], fluorescence [9], spectrophotometry [10], capillary electrophoresis [11–14], and an ISO reference method for meat and meat products [15]. Nevertheless, most of these methods suffer from drawbacks, such as the need of additional derivatization reactions, extensive sample pretreatments, and sophisticated and expensive instrumentations. On the contrary, enzyme amperometric biosensors provide specific, rapid and repetitive assays [16–19] by miniaturizable and low cost transducers [20–22], thus representing valid and attractive alternatives in food quality monitoring and biotechnological and agricultural applications.

The immobilization of L-glutamate oxidase (GLOD) or dehydrogenase (GLDH) onto electrodes for the design of amperometric biosensors has been an area of intense research, where the use of commercial disposable electrode transducers was limited to few cases based on screen printed carbon electrodes and GLDH [23].

Because GLDH biosensors require the use of NAD+ as a cofactor and a proper mediator that have to be integrated in the detection scheme, biosensors are usually based on GLOD [23–34], where the concentration of L-Glu is monitored by measuring the production of hydrogen peroxide from the GLOD-catalyzed oxidation mediated by oxygen or the oxygen consumption [35].

However, biosensors should fulfil several requirements to operate in complex matrices as foodstuffs. They must be robust, interference- and fouling-free, easy to use, and possess a suitable linear range of response. Then, to get these requirements, some important issues have to be taken into account, also considering that each enzyme, analyte, or electrode material shows peculiar chemical properties that need the tailored development of specific fabrication procedures, even when already known processes are used [19,36]. Therefore, in the development of sensitive, selective and accurate biosensors, an efficient enzyme immobilization and a proper selection of the transducer electrode material are mandatory. In fact, the electrode material can affect, on one hand the oxidation of hydrogen peroxide and, on the other hand, the oxidation of the analyte itself and interferents, with a consequent loss in selectivity and electrode fouling, the latter determining a concomitant deterioration of the biosensor sensitivity [36]. Concerning the enzyme immobilization, a right procedure have to be adopted in order to improve the biosensor sensitivity and stability, in particular when screen printed electrodes are used as the transducer [20]. Recently some biosensors [35,37–39] for L-Glu detection in foodstuffs have been proposed, but the above mentioned requirements have not been completely met.

In the present work an accurate first generation L-Glu biosensor has been developed for the first time onto low-cost commercial disposable screen printed platinum electrodes (SPPtEs). The L-Glu biosensor sensitivity, selectivity and stability have been optimized in terms of electrode material, permselective film thickness (overoxidized polypyrrole, PPYox), and enzyme immobilization approach based on bovine serum albumin (BSA) and glutaraldehyde. The L-Glu biosensor has been characterized in terms of performance parameters, such as calibration curve, sensitivity, linearity, LOD, LOQ, precision, accuracy, and selectivity toward the common interferents. The biosensor performances were also compared with those of other GLOD biosensors, developed for the monitoring of foodstuffs. Finally, the potential of the L-Glu biosensor has been also tested by the determination of L-Glu content in different kind of foodstuffs.

2. Materials and methods

2.1. Chemicals

GLOD recombinant from Cosmo Bio Co., LTD (Tokyo, Yapan), BSA and glutaraldehyde (GLU) from Sigma Chemical Co. (St. Louis, MO, USA), and all other chemicals from Mollinckrodt Baker (Phillipsburg, NJ, USA) were used as received. Pyrrole (Aldrich, Steinheim, Germany) was purified by vacuum distillation and stored in the dark at −20 °C until use.

2.2. Real samples

Stock cubes, ketchup and Parmigiano Reggiano cheese were purchased from a local store. An appropriate method of extraction was adopted for each type of foodstuff sample. In particular, stock cubes were crushed using a mortar and 0.2 g of product were sonicated with 20 mL of phosphate buffer salinum (PBS) at pH 7 for 10 min, and the final solution was filtered and diluted 1:10 before analyses. The procedure for the preparation of ketchup extracts consisted in the sonication of 0.2 g of sample with 5 mL of PBS, and the final solution was centrifuged, filtered and diluted 1:10 before analysis. The extracts of Parmigiano Reggiano cheese were obtained processing 3 g of sample with 10 mL of 1 M trifluoroacetic acid solution. After 10 min of sonication, 8 mL of PBS were added and the final solution was centrifuged, filtered and diluted 1:25 before analyses.

2.3. Apparatus

Electrochemical measurements were carried out with a CHI620A potentiostat controlled by the software version 2.07 (CHI Instruments, Inc., Austin, TX, USA). FIA measurements were carried out by a Minipuls 3 peristaltic pump (Gilson, Villiers Le Bel, France) and a six-way low pressure injection valve (Rheodyne mod. 5020, Cotati, CA, USA), equipped with a loop of 110 μL. Experiments with bulk Pt dual working electrodes (EG&G Princeton Applied Research, Princeton NJ, USA; 3 mm diameter, 1 mm electrode gap) were accomplished in a conventional thin-layer electrochemical cell (EG&G Princeton Applied Research, Princeton NJ, USA) by using a thin-layer flow cell gasket of 255 μm thickness. The carrier electrolyte consisted of a pH 7 (I = 0.05 M) phosphate buffer solution (PB), and the detection potential of +0.7 V was quoted versus an external Ag/AgCl (3 M Cl^-) reference electrode. A wall-jet electrochemical cell (DropSens, Asturias, Spain, ref. DRP-FLWCL-P-100806) was used for the experiments with SPPtEs (DropSens, ref. DRP-550, 4 mm diameter) that were carried out in PBS at pH 7, by using the internal reference electrode.

2.4. Electrosynthesized polymer modified electrodes

Bulk Pt dual working electrodes were pre-treated as described in our previous work [40], while SPPtEs were used as received. PPY films were electrosynthesized at +0.7 V in a solution of 10 mM KCl and 0.4 M pyrrole. The deposition charge was 38.15 mC and 93.28 mC for bulk and disposable electrodes, respectively, corresponding to a film thickness of about 1.2 μm. Pt/PPY and SPPtEs/PPY were overoxidized by using a previously adopted procedure [40].

2.5. Biosensor preparation

Enzyme immobilization onto Pt/PPYox and SPPtEs/PPYox modified electrodes was carried out by three different procedures (see Table 1) by using the following solutions: I) 1 mg of GLOD in 50 μL of PB; II) 7 mg of BSA with 12.5 μL of 2.5% GLU solution (25% glutaraldehyde solution diluted 1:10 with PB) and 100 μL of PB; III) 2.5% GLU solution; IV) 1 mg of GLOD, 3 mg of BSA, 12.5 μL of 2.5% GLU solution, and 50 μL of PB.

Each solution was dropped onto the Pt/PPYox and SPPtEs/PPYox modified electrodes according to the sequence displayed in Table 1. Afterwards, the electrodes were left for 30 min at room temperature for each step of deposition.

3. Results and discussion

3.1. Optimization of the enzymatic immobilization

Considering the possible involvement of lysine moieties that could be present in GLOD active site, the use of BSA can prevent the denaturation of the biocomponent during the entrapment by co-crosslinking. However, also the mechanical stability of the enzyme entrapping gel could depend on the number of lysine moieties either of BAS or of the GLOD [36]. Moreover, for the amperometric biosensors, the sensitivity of response depends strictly on both the enzyme loading and the electrode material (naked or modified with polymers), the latter being the substrate onto which the enzyme immobilization is carried out. For these reasons, two types of PPYox modified electrodes, bulk Pt and disposable SPPtEs, were tested in terms of sensitivity of response and anti-interference properties, using different mix of enzyme, BSA and GLU in the immobilization step. On the basis of previous studies [36,40] and preliminary experiments, Pt has been selected as the electrode material, because it guarantees a minimization of the direct L-Glu response with respect to that of hydrogen peroxide, also allowing the efficient preparation of the PPYox permselective film. Since the electroactivity of screen printed electrodes is quite different from that of bulk metal electrodes [20,21], PPY electropolymerization onto SPPtEs was optimized in terms of deposition charge. In order to obtain a film thickness of about 1.2 μm, which is a good compromise in terms of anti-interference properties, response time and mechanical stability of the relevant biosensor [19], a higher deposition charge of 740 mC cm^{-2} was necessary with respect to that of 537 mC cm^{-2} used for bulk Pt electrodes [36]. GLOD immobilization was carried out by means of three different procedures (see Table 1 in the experimental section) based on different combinations of the enzyme, BSA and GLU. Different concentrations of standard L-Glu solutions and a mix of interferents (100 μM ascorbic acid, 100 μM cysteine, 200 μM paracetamol and 500 μM gallic acid) were injected to evaluate the sensitivity of response and the anti-interferent properties of each biosensor prepared with the procedure proposed.

Disposable biosensors (SPPtE/PPYox/GLOD-GLU-BSA) prepared by the procedure A showed the best sensitivity (0.0183 ± 0.0009 A M^{-1} cm^{-2}, n = 6), also with respect to that obtained by using bulk Pt electrodes (0.0127 ± 0.0006 A M^{-1} cm^{-2}, n = 6). This result is due to the highest amount of BSA used in the procedure A, confirmed by a strong decrease of sensitivity by decreasing the BSA in the GLOD immobilization procedure. The biosensor sensitivity reached the lowest value (0.00240 ± 0.00012 A M^{-1} cm^{-2}, n = 3) by the procedure B, where no BSA was used. A similar trend was observed for the PPYox modified bulk Pt electrodes, suggesting that the fabrication procedure is not affected by the electrode material, but by the involvement of the lysine groups of GLOD active site in the crosslinking process, modulated by the presence of BSA. Concerning the anti-interferents properties (see Table 2), similar results were obtained with the lowest overall absolute bias of 46 μM displayed by the disposable biosensor obtained with procedure A. In particular, paracetamol was not detected while ascorbic acid, cysteine and gallic acid showed a bias of 11.0 ± 0.5, 8.0 ± 0.7 and 27.0 ± 0.6 μM, respectively. Similar results were obtained with the Pt/PPYox modified electrode. The differences in the anti-interference characteristics showed by the three immobilization procedures investigated are due either on a different response sensitivity, also affecting the absolute bias and depending on the amount of the active enzyme in the co-crosslinked gel, or the barrier properties towards interferents showed by the gel itself. The SPPtE/PPYox/GLOD-GLU-BSA biosensors obtained by the procedure A, showing the best performances in terms of sensitivity and and anti-interferent properties, were used in the following flow injection analysis (FIA) characterization.

3.2. Biosensor characterization by FIA

SPPtE/PPYox/GLOD-GLU-BSA biosensors obtained by the procedure A were characterized in terms of linearity, LOD, LOQ and stability of response by FIA experiments.

Flow rate is an important parameter affecting both the sampling rate and the biosensor response, the latter being the result of three main kinetic steps (the electrode kinetic is considered fast): L-Glu diffusion from the bulk solution to the electrode, the enzyme reaction, and the H_2O_2 diffusion from the electrode to the bulk solution. The flow rate dependence of the biosensor response was assessed in the range 1.0–0.2 mL min^{-1} by measuring the peak current of a 0.5 mM L-Glu standard solution. The peak currents showed a marked decrease from 0.1 to 0.5 mL min^{-1} and then leveling off at 0.75 mL min^{-1} (10% of the signal at 0.1 mL min^{-1}). This behavior is likely due to both an increase of L-Glu residence time in the flow cell, which behaves as an enzyme reactor where the enzyme reaction is the rate-determining step, and a higher removal of the H_2O_2 produced by the enzyme layer of the modified electrode. Therefore, also considering that the response of electroactive interferents decreases by lowering the flow rate, as the best compromise, in terms of sample throughput, sensitivity and selectivity, a flow rate of 0.5 mL min^{-1} (20% of the signal at 0.1 mL min^{-1}) has been used for all the FIA experiments.

Fig. 1A shows typical FI peaks of L-Glu standard solutions at different concentrations, obtained at a SPPtE/PPYox/GLOD-GLU-

Table 1
- Volumes of solution used in the GLOD immobilization by three different procedures.

Enzymatic immobilization procedure	solution I (GLOD 0.02 mg μL^{-1})	solution II (BSA 0.062 mg μL^{-1} + GLU 0.28%)	solution III (GLU 2.5%)	solution IV (GLOD 0.016 mg μL^{-1} + BSA 0.048 mg μL^{-1} + GLU 0.5%)
			(μL)	
A	2.5	5.0	–	–
B	2.5	–	5.0	–
C	–	–	–	3.0

Table 2
- Mean sensitivity and overall absolute bias of disposable biosensors prepared with different enzyme immobilization procedures.

Enzymatic immobilization procedure	Mean Sensitivity (n = 3) (A M^{-1} cm^{-2})	Mean Overall Absolute Bias (n = 3) (μM)
A	0.0183 ± 0.0009	46.0 ± 2.0
B	0.00240 ± 0.00012	326 ± 16
C	0.0079 ± 0.0004	1186 ± 59

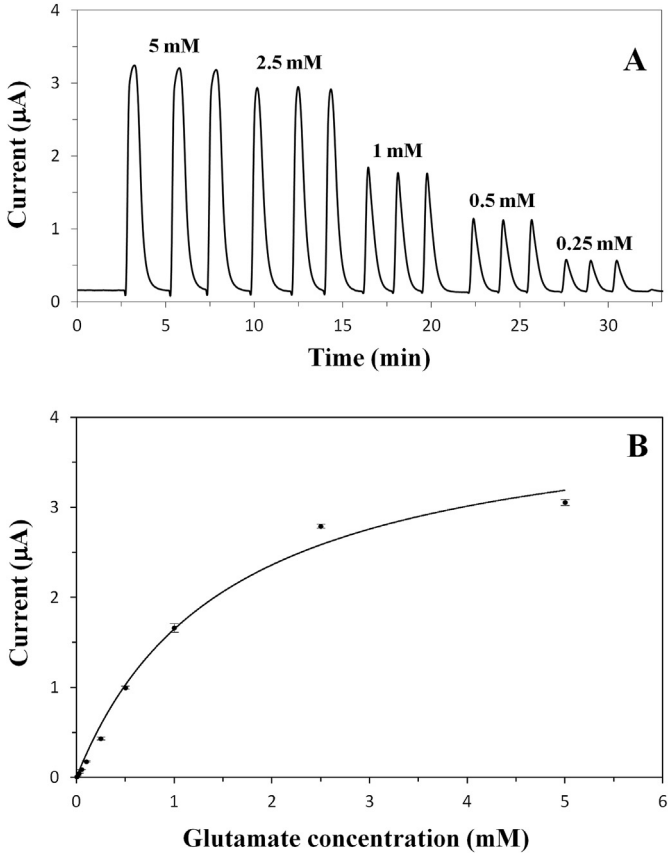

Fig. 1. A: FI peaks of glutamate standard solutions obtained at a disposable SPPtE/PPYox/GLOD-GLU-BSA biosensor. **B**: Calibration curve and plot of the non-linear regression fitting to the Michaelis-Menten law.

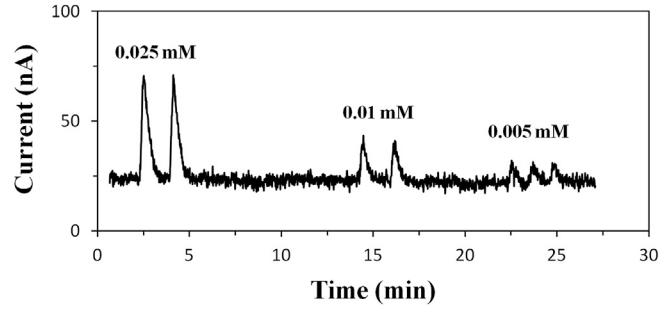

Fig. 2. FI peaks of glutamate standard solutions at low concentrations obtained at a disposable SPPtE/PPYox/GLOD-GLU-BSA biosensor.

BSA biosensor, while in the panel B is displayed a typical calibration curve obtained in the concentration range 0.005–5 mM. Experimental data (current responses vs. L-Glu concentrations) were submitted to a non-linear regression fitting to the Michaelis-Menten law, and the relevant fitting curve, expressed by the regression equation I = (4.17 ± 0.23) C/(1.53 ± 0.21 + C) ($\chi^2 = 0.0904$, n = 10, three replicates each) is also plotted in Fig. 1B. The fitting result clearly indicates that the biosensor response trend follows the Michaelis–Menten law, and that from the regression equation accurate enzyme apparent kinetic parameters (I'_{max} and K'_m) can be obtained for the immobilized enzyme. A K'_m of 1.53 mM higher than that of the enzyme free in solution (K_m = 0.2 mM from the data sheet of supplier) can be explained in term of a limitation to substrate diffusion [40] into the enzyme entrapping gel.

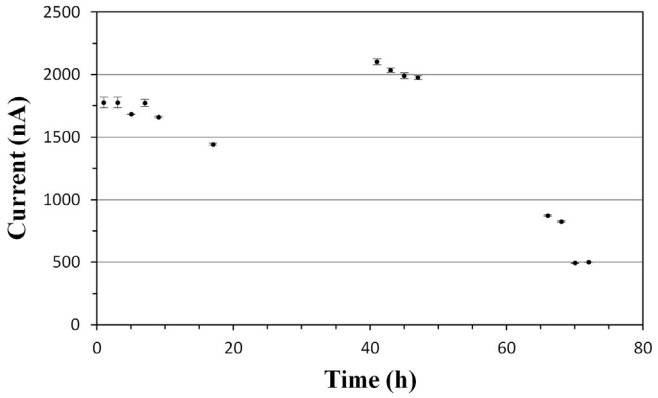

Fig. 3. Long-term operational stability of a SPPtE/PPYox/GLOD-GLU-BSA biosensor in a FI system obtained by repetitive injections of glutamate 1 mM in a 4-day period of time.

The biosensor response was linear in the range 0.005–1.0 mM (linear regression equation: I(μA) = 1.71 C(mM)+0.017, $r^2 = 0.992$, n = 8 three replicates each) with a dynamic range up to 2.5 mM. LOD and LOQ of 1.8 μM and 5.4 μM were obtained from the linear regression equation applied to the biosensor responses obtained in the range 0.005–0.1 mM (n = 5) at low concentrations (see Fig. 2), calculated as 3.3 sy/slope and 10 sy/slope, respectively, where sy is the standard error of y-intercept.

Under the above operational conditions, a sampling rate of 30 s h^{-1} was achieved. In Fig. 3 is displayed the long-term stability of the disposable biosensor that has been assessed in FIA by the mean response of a 1 mM L-Glu standard solution injected several times during each working day, without dismantling the electrochemical cell and leaving the biosensor with a constant potential applied to the working electrode. Biosensor was stable up to 50 h under continuous operational conditions (over 400 injections), then the response decreased up to 25% of the initial signal, owing to a probable detachment of the enzyme entrapping layer or the enzyme denaturation during the FI analysis. However, the operational stability of the biosensor developed onto a disposable electrode device resulted suitable for accurate and rapid routine analyses of L-Glu in complex real food matrices.

On the basis of an inter-device reproducibility below 5%, calculated from the response sensitivities of six different SPPtE/PPYox/GLOD-GLU-BSA biosensors, the fabrication procedure seems to be very attractive for mass-scale production. Table 3

Table 3
- Comparison of performances of glutamate oxidase (GLOD) based amperometric biosensors for glutamate analysis in foodstuffs.

Biosensor configuration *Enzyme source* (Analysis operational mode)	Sensitivity (mA M^{-1} cm^{-2})	Linearity (mM) LOD/LOQ (μM)	Interferents (concentration tested/bias)	Stability	Real sample	Reference
O_2 electrode/Polycarbonate/GLOD-GLU-BSA *Streptomices* sp. (Batch)	-*	0.059–1.18 n.d./n.d.	n.d.	16 tests in 48 days storing at 4 °C when not in use.	Soy sauce, tomato sauce, chicken Thai soup, chili chicken	[35]
GC/AuNanowires-MultiwalledCarbonNanotubes-Chitosan/GLOD *Streptomices* sp. (Flow Injection)	1.88	0.01–0.1 1.2/n.d.	n.d.	98.5% at day 16th in a day-to-day stability test: triple injections a day. 91.3% after 15 repetitive injections, then a gradual decrease over time.	Rosdee, Kanorr, Takumi sauce, soy sauce, fish sauce, chicken essence	[37]
Pt/poly(1,3-diaminobenzene)/GLOD-poly(vinylferrocene)-poly(ethylenglycole)/dyalisis membrane *Recombinant* (Flow Injection)	0.134	0.5–8.0 n.d./n.d.	Ascorbic acid (0.5/0.06 mM), cysteine (0.5/0.11 mM), L-aspartic acid (0.5/0.06 mM), other amino acids (0.5/n.r. mM)	Stable up to 4 days in a day-to-day stability test: 50 injection a day and storing the biosensor at 4 °C when not in use. After 16 days, the response was 60% of the initial value.	15 different soy sauce products	[38]
Pt–C/polycarbamylsulfonate-hydrogel-polyethylenimine-GLOD *Streptomices* sp. (Batch)	98.8	0.1–1.6 1.01/n.d.	Several amino acids (1mM/relative interference in the range 0/9%) and metal ions (1mM/relative interference in the range −27/+5%)	About 90% for 2 weeks in a day-to-day stability test: one test a day and storing the biosensor in moisture condition at room temperature. After 30 days, the response was about 60% of the initial value.	Knorr seasoning, 4 different soy sauce products	[39]
SPPtE/PPYox/GLOD-GLU-BSA *Recombinant* (Flow Injection)	18.3	0.005–1 1.8/5.4	Ascorbic acid (0.1/0.011 mM), cysteine (0.1/0.008 mM), paracetamol (0.2/n.r. mM), gallic acid (0.5/0.027 mM)	Stable up to 50 h (over 400 injections) of continuous use, then 25% of initial signal.	Stock cube, ketchup, Parmigiano Reggiano cheese	Present work

* Sensitivity of 0.0697 as O_2 ppm•dl/min•mg.
n.d. = Not determined.
n.r. = No response.

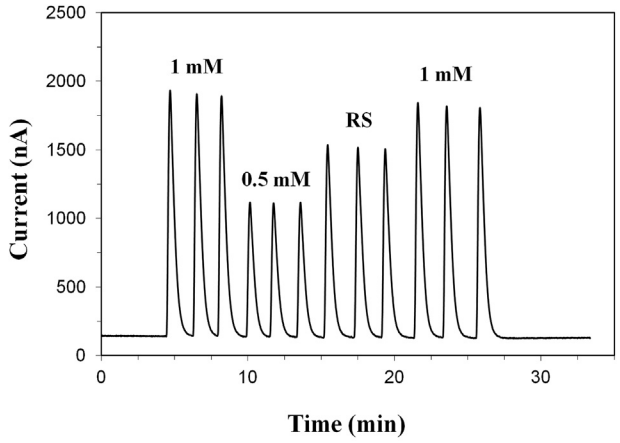

Fig. 4. FI peaks obtained at a disposable SPPtE/PPYox/GLOD-GLU-BSA biosensor for repetitive analyses of a stock cube extract (RS) and standard solutions of glutamate.

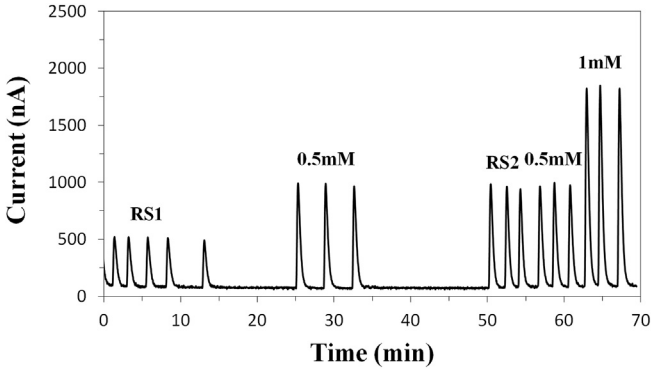

Fig. 5. FI peaks obtained at a disposable SPPtE/PPYox/GLOD-GLU-BSA biosensor for repetitive analyses of ketchup (RS1) and Parmigiano Reggiano cheese (RS2) extracts, and standard solutions of glutamate.

Table 4
- Glutamate contents of real samples and relevant percentage relative standard deviations obtained by a disposable SPPtE/PPYox/GLOD-GLU-BSA biosensor.

Real Sample	Free L-Glu in foodstuffs[a]	Biosensor response[a]	RSD%[b] (n = 3)
Stock cube	10–15	13.5	1.3
Ketchup	>0.250	0.998	0.7
Parmigiano Reggiano cheese	1.20–1.70	1.29	3

[a] Percentage expressed as monosodium glutamate.
[b] Percentage relative standard deviation.

highlights the better performances of the proposed first generation GLOD amperometric biosensor with respect to those of other amperometric glutamate oxidase biosensors for the analysis of foodstuffs, based on more complex detection schemes. Specifically, SPPtE/PPYox/GLOD-GLU-BSA biosensor shows a higher response sensitivity, a higher stability in continuous use, and excellent anti-interferent characteristics. These features make the proposed glutamate biosensor a suitable tool for the accurate monitoring of complex matrices even containing low L-Glu levels.

3.3. Application of biosensor to real samples

The potential of GLOD disposable biosensor was tested by the analysis of L-Glu in real samples, such as stock cube, ketchup and Parmigiano Reggiano cheese. Figs. 4 and 5 show FI peaks of real sample extracts and L-Glu standard solutions. As it can be seen the excellent response repeatability (RSD% < 3%) in the analysis of L-Glu standard solutions, obtained before and after injections of the real sample, evidence the absence of any fouling effect, caused by high molecular weight components (e.g. proteins) normally occurring in foodstuff, and/or the absence of the enzyme denaturation, so proving the biosensor reliability.

In Table 4 are summarized the L-Glu contents for each foodstuff and the relevant relative standard deviations. No significant differences of L-Glu contents were observed (t-test at a 95% confidence level) by the comparison of the results obtained with the biosensor and by a standard reference method. L-Glu levels measured by the biosensor were also in the typical range reported for these foodstuffs. For example, for the stock cube samples, the value of 13.6% obtained with the biosensor is in the range 10–15% reported for different type of stock cubes, then confirming the goodness of the proposed biosensor in the accurate L-Glu monitoring in foodstuffs.

4. Conclusions

The proposed glutamate biosensor was prepared by a tailored GLOD immobilization procedure, through the co-crosslinking with BSA and GLU onto a SPPtE modified with a permselective PPYox film. The enzyme immobilization was optimized by testing different experimental procedures, and the resulting SPPtE/PPYox/GLOD-GLU-BSA biosensors showed a better sensitivity than that of biosensors prepared by using bulk Pt electrodes. The use of permselective PPYox films and the proper electrode material selection allowed the preparation of an anti-interference high performance glutamate disposable biosensor possessing a very good sensitivity, linearity and operational stability, and limits of detection and quantitation in the order of few micromolars. These performances, which are better than the other GLOD amperometric biosensors, permitted accurate glutamate analyses in complex foodstuffs with a very good sample throughput. The results were in excellent agreement with those of a standard reference method, demonstrating that the proposed biosensor is a valid alternative for the rapid and accurate monitoring of glutamate in different kind of complex foodstuffs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Annalisa Mentana: Investigation, Writing - original draft. **Donatella Nardiello:** Validation. **Carmen Palermo:** Investigation, Formal analysis. **Diego Centonze:** Conceptualization, Supervision, Methodology, Writing - review & editing, Visualization.

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