Portugaliae Electrochimica Acta 25 (2007) 173-183

PORTUGALIAE ELECTROCHIMICA ACTA

Electrochemical Evaluations of Glutamate at a Gold Electrode

M. Goreti F. Sales,[*] Cláudia Martins, M. Fátima Barroso, M. Carmo V.F. Vaz, M. Beatriz P. Oliveira, C. Delerue-Matos

Requimte/Instituto Superior de Engenharia do Porto
Rua Dr. António Bernardino de Almeida nº 431, 4200-072 Porto, Portugal

Abstract
The voltammetric behaviour of glutamate at a gold electrode was studied by means of cyclic voltammetry. From 2.6 to 11.5 pH values, glutamate originated a single cathodic peak. Potential of the peak, E_p, was -0.8 V, and it was independent from pH. The reduction of glutamate was found irreversible and mainly controlled by diffusion.
An analytical approach for analysis of glutamate by means of square wave voltammetry was developed. Plots of current *versus* concentration presented a linear behaviour from 2.5×10^{-4} to 2.7×10^{-3} M. The detection limit was 6.3×10^{-5} M. Interference from compounds co-existing with glutamate in food was negligible, and in favour of an application of the proposed method to the analysis of real samples. Determination of glutamate in pure solutions resulted in acceptable deviation from the stated concentration. Relative errors ranged -2.5 to +1.6 %.

Keywords: glutamate, gold electrode, voltammetry, flavour enhancer, food.

Introduction

Glutamate is a well known flavour enhancer that has been used for several decades to enhance the flavour and taste of food throughout the world. Food sauces, instant soups and traditional fermentation products such as soy sauce contain relatively high levels of glutamate [1,2]. Ingestion of glutamate has been however correlated to adverse reactions recognized as the Chinese restaurant syndrome, also referred to as the monosodium glutamate symptom complex, occurring in certain groups of the population when food with high amounts of

[*] Corresponding author. E-mail address: mgf@isep.ipp.pt

this flavour enhancer is ingested. Thus, to ensure consumer's safety the levels of glutamate in commercial food products must be controlled.
Many analytical methods are described in literature for determination of glutamate [3-14]. Commercially available and the most common method for determination of glutamate is the end-point colorimetric method [15], which is considered to be time-consuming and laborious. Other methods also based in optical techniques are presented in literature (see table 1). They are supported mostly by enzymatic procedures to enable high selectivity. However, this favourable feature is coupled to increased costs and short viability of application within time.
Alternative strategies in literature are based in HPLC procedures (see table 2). Different kind of detection systems are coupled to the chromatographic separations. They regard the measurement of either an optical or an electrical property. Inherent methods are precise, accurate and robust. In addition, they are also of high cost for a routine application.
Other methods published in literature are electrochemical biosensors [16-20]. These devices provide selective readings at low levels of glutamate. Since they are not long lasting they turn out expensive and unsuitable for routine applications.
Linking the high levels of glutamate in most foods to the necessity of routine measurements, a simple method of low cost and quick response would be appreciated. This could be applied to routine trial evaluations meant to ensure that levels of glutamate are always below the maximum admissible amount. Thus, this work aims to study the voltammetric response of glutamate at gold electrode and to monitor its suitability at routine analytical applications.

Experimental

Apparatus

All voltammetric measurements were performed using a 663 VA Metrohm cell containing a gold working electrode (GE) (0.5 mm diammeter), a glassy carbon rod as counter electrode (Metrohm 6.1247.000) and an Ag/AgCl (KCl 3.00 M) reference electrode (Metrohm 6.0728.000) attached to an Autolab PSTAT 10 potentiostat/galvanostat (Ecochemie) running with model GPS.
GE were mechanically cleaned before each experiment by polishing their surfaces with a polishing kit (Metrohm 6.2802.010), first with α-Al2O3 (0.3 μm) until a shining surface was obtained, and after with only water.
pH measurements were made with a decimilivoltammeter Crison®, pH meter, GLP 22, connected to a CWL/S7 combined glass electrode. The electrode was calibrated with commercially available buffer reference solutions.

Table 1. Optical methods for glutamate determination in food and biological samples.

Technique	Experimental guidelines of the method	Linearity range (μM)	Limit of detection (μM)	Sample	Ref.
UV/Vis	L-Glutamate desidrogenase transforms glutamate in 2-oxoglutarate and NAD+. The later is reduced to NADH while the former reacts with D-4-hydroxyphenylglycin to form 4-hydroxybenzoylformate after catalysis by D-phenylglycine aminotransferase. NADH and 4-hydroxybenzoylformate are quantified by monitoring absorbance readings at 340 nm.	0.2 – 20	0.14	Fish and soy sauces	3
Luminescent	Similar to previous one, but carried out in flow media. Fluorimetric detection is made at λ_{em} of 460 nm after λ_{exc} of 340 nm.	2.5 – 50	0.4	Fish and other sauces	4
	Glutamate reacts with glutamate oxidase to form H_2O_2; this product reacts with luminol when peroxidase is present, producing a maximum emitted radiation at 280 nm; carried out in flow media.	0.020 – 50	0.010	Serum	5
	Fluorescent reaction with "Amplex red" and H_2O_2 after enzymatic oxidation of glutamate in the presence of oxygen; λ_{exc} 530 nm and λ_{em} 590 nm.	0.005 – 1.25	0.005	Food	6
	Glutamate is oxidized by NAD^+ thus producing the NADH. This reduced form is made react with resazurin to obtain a fluorescent product (λ_{exc} 530 nm and λ_{em} 590nm).	0.02 – 1.25	0.020		

Table 2. HPLC based glutamate determinations in food and biological samples.

Detection	*Stationary phase*	*Mobile phase*	*Other operating conditions*	Linearity range (μM)	Limit of detection (μM)	Sample	Reference
MS	5 μm inertsil ODS-2 (150×4.6 mm i.d.)	10% acetonitrile in ammonium acetate 100 mM	Flow-rate: 0.9 mL/min Retention time < 2 min.	-	40	Mouses	7
CD	5 μm Econosil CN (250×4.6 mm i.d.)	Water/acetonitrile/tetrahydrofuran (77:20:3) with 1 mM of perchloric acid or 1 mM of trichloroacetic acid	Retention time < 10 min.	0-4000	-	Starchy food	8
FL	4 μm C18 (150×3,9 mm i.d.)	Gradient procedures of (A) 120 mM potassium phosphate and (B) 120 mM potassium phosphate/ acetonitrile/methanol (46:18: 18)	λ_{exc} 340 nm; λ_{em} 425 nm	40-200	80	Mouses	9
	5 μm C18 (250× 4.6 mm i.d).	sodium hydrogenophosphate 0.05 M, hexansulphonic acid 0.01M, triethylammine trichloroacectic acid 0.0072M	Retention time < 5 min. λ_{exc} 340 nm; λ_{em} 425 nm.	40 – 200	8	Drugs	10
	5 μm C18 (50×4 mm i.d.) (100×3.2 or 1 mm i.d.)	-	Retention time < 5 min. Chemical derivatization λ_{exc} 330 nm; λ_{em} 418 nm.	-	-	Microdialysis	11
	4 μm AccQ.Tag (150×3.9 mm i.d.) or C18 (20 × 3,9 mm i.d.)	Gradient with acetronitrile and AccQ.Tag.	Retention time < 22 min. λ_{exc} 250 nm; λ_{em} 395 nm	2.5-200	-	Infant cereals	12
UV	For free aminoacids (30×3.9 mm i.d.)	Gradient procedure of (A) sodium acetate 70 mM (pH 6.5) and acetonitrile and 0,025% EDTA, and (B) acetonitrile/water/methanol (45:40:15)	Retention time < 5 min Chemical derivatization λ 254 nm	25-2500	80	Stomach mucosa	13
	5 μm LiChorospher 100 RP-18 (250 × 4 mm i.d.)	100 mM ammonium acetate with 10% acetonitrile	Flow-rate: 0.9 mL/min Retention time < 2 min Chemical derivatization λ 254 nm	-	2	Not applyed	14

Reagents and solutions

Glutamate was purchased from Fluka and used without further purification. All other chemicals were Merck pro analysis grade and all solutions were prepared using purified water (conductivity < 0.1 μS cm^{-1}) obtained from a Barnstread E-pure 4 system.

Voltammetric assays were carried out in KNO_3 electrolyte with an ionic strength of 5×10^{-1} M. Electrolytes of different pH values were prepared after suitable addition of HCl or KOH solutions to a KNO_3 electrolyte solution. Solutions ranging 2.6 and 11.5 pHs and of 5×10^{-1} M ionic strength were obtained after this.

Stock solutions of about 1.00×10^{-1} M in glutamate were prepared by dissolving an exact amount of the pure compound in water. This solution was stored at +4 ºC in the dark to prevent chemical alterations and considered stable for one week. Working standard solutions were prepared daily by diluting the previous stock solution with a selected supporting electrolyte just before use.

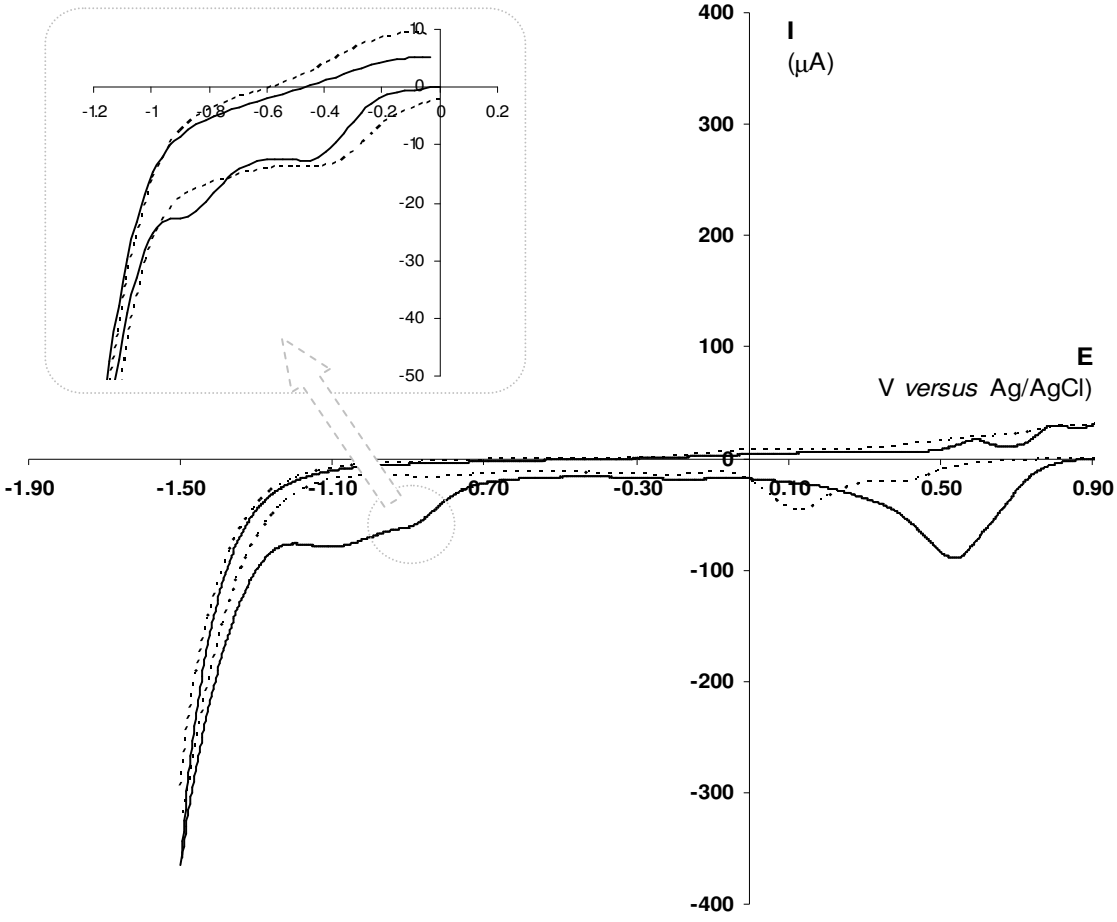

Figure 1. Cyclic voltammograms of KNO_3 supporting electrolyte (---) and 2.0×10^{-3} M glutamate (—) at a GE, with a scan rate of 50 mV/s. Inset: restricted scanning of the same supporting electrolyte (---) but with decreased glutamate concentration (—) of 6.0×10^{-4} M.

Procedures

Supporting electrolyte (20.0 mL) was placed in the electroanalytical cell. The solution was deoxygenated before analysis by purging with purified nitrogen for 2 minutes. A known volume of stock glutamate standard solution was transferred to the electrochemical cell and this solution was purged with purified nitrogen for 30 seconds. This procedure also ensured homogenisation of the resulting solution.

Electrochemical studies of glutamate at a gold electrode were carried out by means of Cyclic Voltammetry (CV) and Square Wave Voltammetry (SWV). The electrode surface was polished between two consecutive scans. Voltammograms were recorded in quiescent solution. After a quiescent period of 5 s the potential scan was started. All measurements were made at room temperature.

Results and discussion

The electrochemical behaviour of glutamate at the gold electrode was investigated by CV and SWV over the pH range 2.6 to 11.5. Trial experiments in electrolytes of different nature showed best-defined peaks for KNO_3 supporting electrolytes. Hence, this supporting electrolyte was selected to carry out all subsequent experiments. All pH changes were made after addition of HCl or KOH solutions. These were selected to avoid addition of foreign ionic species to the supporting electrolyte.

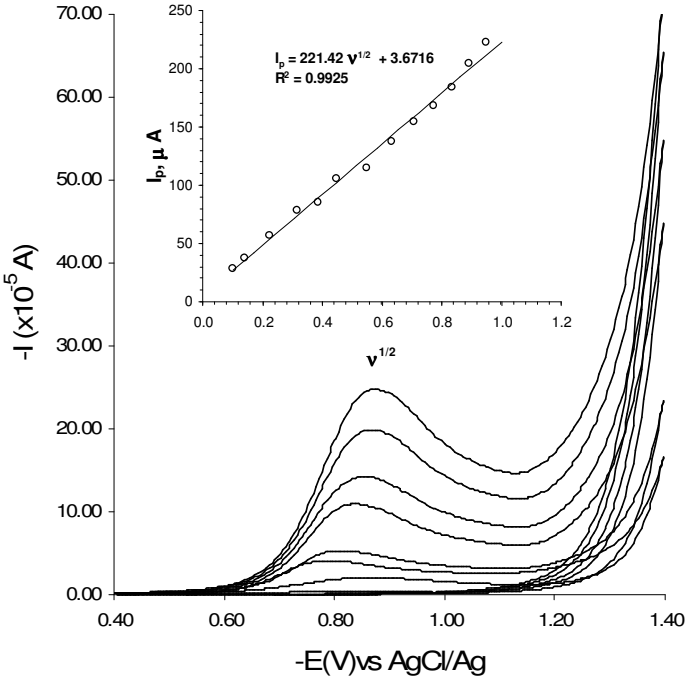

Figure 2. Cyclic voltammograms of a GE in 2.2×10^{-3} M glutamate solution using the scan rate 10, 20, 100, 200, 400 and 600 mV/s. Inset: linear correlation between i_p and the square root of the scan rate.

Cyclic Voltammetry

CV was performed from −1.5 to +1.5 V with the scan rate varying from 10 to 600 mV/s. Voltammograms of glutamate show a single peak in aqueous solution (Fig. 1) at all pH values investigated. This reduction peak occurs at -0.80 V, applied over a gold surface *versus* an AgCl/Ag reference electrode. Absence of any anodic peak on the reverse scans indicates the irreversible nature of the electrode reaction. This irreversible nature is recorded for all scan rates and all pHs studied.

The influence of the scan rate on the peak current (i_p) was studied within the range 10 and 600 mV s^{-1} (Fig. 2). The cycles carried out within the increased values of scan rate produced a linear relationship with the square root of the scan rate, indicating that the process at the surface of the electrode was mainly controlled by diffusion.

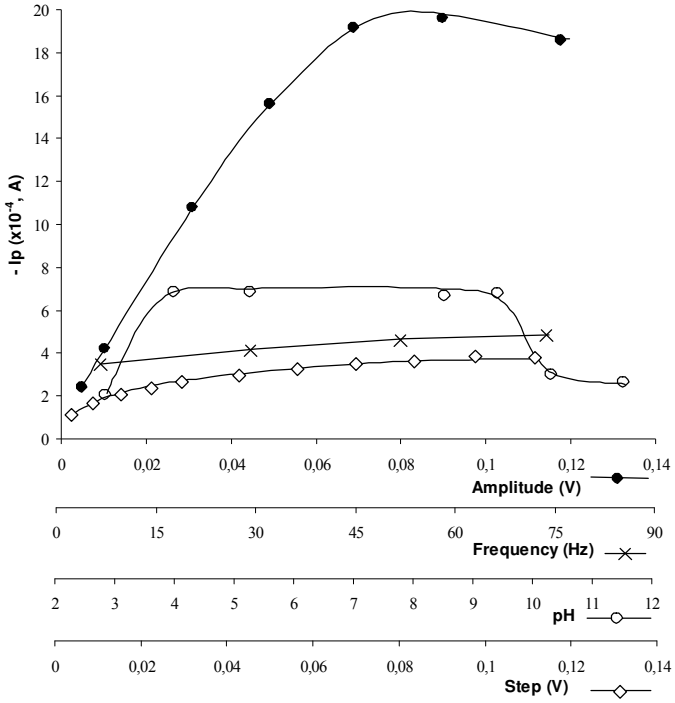

Figure 3. Influence of pH (keeping frequency, step and amplitude at 30 Hz, 0.03 V and 0.09 V, respectively), step (30 Hz frequency, 0.09 V amplitude and pH 8), amplitude (30 Hz frequency, 0.105 V step and pH 8), and frequency (0.105 V step, 0.05 V amplitude and pH 8) on the SWV reduction peak current (Ip) of 2.0×10^{-3} M glutamate at gold electrode.

Square Wave Voltammetry

SWV is a technique that enables simultaneous inspection of both reduction and oxidation processes and hence provides an insight into the mechanism of the electrode reaction. Forward and backward components of the square wave voltammetric response show a single cathodic peak, thus confirming the irreversible nature of the electrochemical reaction.

Voltammograms indicate that i_p depends on the pH of the medium (Fig. 3). Glutamate presented eletroreduction activity at all pH values, but higher $-i_p$ are recorded within 4 to 10. A value of pH 7.6 was selected for subsequent studies.

Considering that SWV response depends markedly on instrumental conditions, several parameters of concern were optimized unvaryingly. Pulse amplitude, step and frequency were considered for this purpose. Main results of this study are presented in Fig. 3 and report variations of peak height for a 2.0×10^{-3} mol L^{-1} glutamate solution.
Frequency was varied from 10 to 70 Hz. Reducing peak currents increased with the frequency until 70 Hz. After 30 Hz the peak of glutamate became deformed. The pulse amplitude was varied between 50 and 120 mV. The peak current increased linearly up to 70 mV and then remained more or less constant. The step was studied in the range of 10 and 80 mV and the peak current improved until to a maximum of 70 mV. As in the frequency study, after 30 mV the peak of glutamate became deformed. Thus, experimental conditions selected for SWV subsequent experiments are 30 Hz for applied frequency, 70 mV for pulse amplitude and 30 mV for step.

Main analytical features
Electroanalytical procedures were established for the SWV determination of glutamate. Table 3 shows the main analytical features obtained after calibrations carried out with previously selected experimental conditions and with standard solutions of $(0.1 - 3)\times10^{-3}$ M.

Table 3. Main analytical features of SWV glutamate measurements.

Analytical feature	*GE*
pH	7.6
Ep (V)	-0.80
Linear range ($\times10^{-4}$ M)	2.5 – 27
Slope ($\times10^{-4}$ A/mM)	0.34 ± 0.01
Intercept ($\times10^{-5}$ A)	4.01 ± 0.1
Correlation coefficient	0.9965
LOD ($\times10^{-4}$ M)	0.63
LOQ ($\times10^{-4}$ M)	1.9

The SWV response showed a linear behaviour for glutamate standard solutions ranging 2.5×10^{-4} to 2.7×10^{-3} M. Limits of detection (LOD) and quantification (LOQ) are $6.3\text{x}10^{-5}$ and $1.9\text{x}10^{-4}$ M, respectively. These were calculated from the calibration plots using the equations: LOD = 3s/m and LOQ = 10s/m, where s is the standard deviation of the intercept and m is the slope of the calibration plot. Repeatability was assessed by performing eleven replicate measurements of 4.0×10^{-4} M glutamate solutions. Relative standard deviation was of 3.2 %, thus suggesting that the method is of good precision.

Table 4. Interfering effect of some coexisting compounds.

Interference		*Current (μA)*		*Recovery*
Compound	*% of Glutamate*	*Without*	*With*	*(%)*
Mannitol	10		26.4 ± 1.3	99.6
	100	26.6 ± 0.3	25.6 ± 2.7	96.3
	1000		24.5 ± 0.4	92.24
Sucrose	10		27.0± 1.5	102.1
	100	26.5± 1.8	28.3 ± 1.2	106.9
	1000		28.7 ± 1.7	108.5
Sorbitol	10		25.6 ± 1.1	91.3
	100	26.6 ± 2.2	33.2 ± 1.2	108.1
	1000		31.4 ± 0.8	110.2
Barium chloride	10		23.4 ± 0.6	93.7
	100	25.0 ± 1.0	24.0 ± 0.2	96.0
	1000		24.8 ± 0.9	99.3
Calcium nitrate	10		29.4 ± 0.8	103.7
	100	28.3 ± 1.12	28.4 ± 1.5	100.2
	1000		28.7 ± 1.9	101.3
Tartrate sodium	10		30.9 ± 1.4	109.5
	100	28.6 ± 0.3	26.5 ± 0.7	93.9
	1000		29.2 ± 2.3	103.3
Niquel nitrate	10		47.4 ± 0.2	149.8
	100	31.4 ± 0.2	45.1 ± 35.6	143.6
	1000		124.7 ± 43.4	397.2
Copper (II) nitrate	10		47.4 ± 0.2	149.8
	100	31.4 ± 0.2	45.1 ± 35.6	143.6
	1000		124.7 ± 43.4	397.2
Lead nitrate	10		108.2 ± 7.1	381.4
	100	28.4 ± 0.4	750.3 ± 11.6	2644.5
	1000		2663.2 ± 77.8	9396.4

Effect of coexisting species

Glutamate is added to food to enhance flavour. The maximum level used for this purpose is about 10 g/Kg of sample. As possible interfering compounds for its determination in food samples many inorganic and organic compounds could be considered. Therefore, only some species that are susceptible of electrochemical reduction have been selected for this study.

Different molar ratios of glutamate and possible interfering compounds (10:1, 1:1 and 1:10, respectively) have been evaluated. Main results are presented in table 4 and point out negligible interference form most studied species. Only Ni, Cu(II) and Pb(II) seem to interfere significantly. This effect is of reduced importance

because these are not common among food matrices, especially at the molar levels in study.
In case the analytical method is used to trial levels of glutamate and ensure that no upper limit is disrespected, results point out that all species in study are of negligible interference. No compound provides a consistent and significant decrease at glutamate concentration when co-existing with the analyte.

Analytical application
Glutamate was successfully determined in pure solutions after calibration by SWV carried out under previously selected experimental conditions. Different concentration levels of analyte ranging about 90.0 to 300.0 mg/L were tried out.

Table 5. Determination of glutamate in spiked solutions.

Glutamate (mg/L)		Recovery (%)	RSD[a] (%)	Relative error (%)
Taken	Found			
93.80	91.44	97.49	12.09	-2.51
155.56	155.83	100.18	3.59	0.18
247.06	250.96	101.58	0.45	1.58
307.32	303.64	98.80	0.76	-1.20

[a] RSD: relative standard deviation.

Main analytical results are presented in table 5. Mean recovery values were always close to 100 %. Plotting glutamate amount taken *versus* found amount gives a linear correlation of slope close to unit (0.9977), a small origin displacement (0.9303), and a squared correlation coefficient of 0.9988.
The overall procedure may take about 15 minutes *per* calibration with seven standard solutions and 3 minutes *per* triplicate measurement of spiked sample. This includes placing the supporting electrolyte at the voltammetric cell and spiking it with the proper amount of standard and/or sample. In case the amount of sample is insufficient an increased volume may be added after as long as the dilution effect is considered. This estimated time would require further evaluation in case carry over is found after contact of the GE with real food matrix. It is expected that each calibration may be used safely at the analyses of at least five samples.
Regarding reagent consumption, each calibration procedure with eight standard solutions required about 20 mL of supporting electrolyte and 2.3 mL of standard glutamate solution of 0.10 M. The supporting electrode is composed of nitrate, potassium and/or chloride. Environmental effect of the effluents produced after the analytical procedure is considered of small concern. They contain mostly phosphate that might find use in agriculture and a small amount of glutamate of about 8 mg.

Conclusions

Electrochemical reduction of glutamate at a gold surface is irreversible and diffusion controlled. The potential of the peak is independent from pH but higher reducing currents are within 4 to 10.

The SWV analytical procedure is fast and simple, of low cost and of small environmental impact, all suitable features when routine measurements are intended. The method also offers advantages in terms long viability of application because it is not enzymatic-based. It was found precise, seems accurate, and is inexpensive regarding reagent consumption and equipment involved. Further studies are required to confirm application of the proposed method to real case studies.

References

1. Y. Xu, *J. Ferment. Bioeng*. 70 (1990) 434.
2. T. Ueki, Y. Noda, Y. Teramoto, R. Ohba, S. Ueda, *J. Ferment. Bioeng*. 78 (1994) 262.
3. E. Valero, F. Garcia-Carmona, *Anal. Biochem*. 259 (1998) 265.
4. J. Chapman, M. Zhou, *Anal. Chim. Acta* 402 (1999) 47.
5. E. Swanepoel, M.M. Villiers, J.L. du Preez, *J. Chromatogr. A* 729 (1996) 287.
6. A.A. Karyakin, E.E. Karyakina, L. Gorton, *Anal. Chem.* 72 (2000) 1720.
7. R. Puchades, L. Lemieux, R.E. Simard, *J. Food Sci.* 54 (1989) 423.
8. C.D. Stalikas, M.I. Karayannis, S. Tzouwara-Karayanni, *Talanta* 41 (1994) 1561.
9. N. Kiba, T. Moriya, M. Furusawa, *Anal. Chim. Acta* 256 (1992) 221.
10. F. Mizutani, Y. Sato, Y. Hirata, S. Yabuki, *Biosens. Bioelectron.* 13 (1998) 809.
11. N. Kiba, T. Miwa, M. Tachibana, K. Tani, H. Koizumi, *Anal. Chem.* 74 (2002) 1269.
12. M.I.P. Oliveira, M.C. Pimentel, M.C.B.S.M. Montenegro, A.N. Araujo, M.F. Pimentel, V.L. Silva, *Anal. Chim. Acta* 448 (2001) 207.
13. T. Yao, S. Suzuki, T. Nakahara, H. Nishino, *Talanta* 45 (1998) 917.
14. C. Janarthanan, H.A. Mottola, *Anal. Chim. Acta* 369 (1998) 147.
15. H.O. Beautler, Methods of Enzymatic Analysis, 3rd ed., vol. VII, Verlag Chemie, Weinheim, FL, 1985.
16. R. Pauliukaite, G. Zhylyak, D. Citterio, U.E.S. Keller, *Anal. Bioanal. Chem.* 386 (2006) 220.
17. A.W.K. Kwong, B. Grundig, J. Hu, R. Renneberg, *Biotech. Lett*. 22 (2000) 267.
18. R. Kurita, H. Tabei, K. Hayashi, T. Horiuchi, K. Torimitsu, O. Niwa, *Anal. Chim. Acta* 441 (2001) 165.
19. L. Bang, W. Tan, *Anal. Chim. Acta* 401 (1999) 91.
20. R.I. Stefan, H.Y. Aboul-Enein, J.F. van Staden, *Sens. Update* 10 (2002) 123.